Dendroecological analysis of the altitudinal shift of larch budmoth outbreaks with climate change in the french Alps

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Società Entomologica Italiana

Accademia Nazionale Italiana di Entomologia

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Università degli Studi di Napoli Federico II

Dipartimento di Agraria

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POSTER SESSIONS

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ECE 2018
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Plenary Sessions

PL001

EAVESDROPPING ON COMMUNICATION AMONG ANTS, BUTTERFLIES, AND PLANTS

Francesca Barbero, Department of life sciences and systems biology, University of Turin, Italy

Abundance, biomass, and diversity of ants are among the richest of the animal kingdom. Several factors concur to their ecological success. For instance, their social organization, based on communication signals which coordinate large numbers of individuals in collective-decision making, as well as the colony plasticity to respond to biotic and abiotic variations. The enemy-free space provided by guarding ants and the fiercely defended resources of the nest have attracted a number of distantly-related organisms, giving rise to a variety of symbioses. These associations range from facultative mutualism to obligate parasitism. Among the latter, one of the best-studied models is the *Maculinea* butterfly-*Myrmica* ant system. After a two-week phytophagous period spent on a species-specific food plant, IV-instar butterfly larvae drop on the ground. The parasites do not actively enter the host ant nests but are carried and adopted by foraging workers by means of chemical and acoustical deception. Reared inside the host colony, the larvae turn into pupae within the nest and eventually the adult emerges walking through the galleries. Here I review behavioral, chemical and vibroacoustic adaptations evolved by the butterfly parasite to exploit its host ants. The circumvention of *Myrmica* communication signals is required to enable *Maculinea* larvae to live undamaged and in close contact with workers for most of their life cycle. Evolutionary significance of vibroacoustic and chemical cues are discussed in the context of multimodal communication. The role of the food plant in underpinning this multitrophic interaction is also taken into account in a community context-dependent study. In such a complex system, assessing bi-level interactions independently is often not conclusive. Conversely, the survey of the overall variation in associated costs and benefits is advisable to fully disentangle co-evolutionary dynamics in multifaceted interactions.

Keywords: Ants, Butterflies, Chemical mimicry, Vibroacoustics

PL002

THE PHYLOGENY OF HEXAPODA AND THE EVOLUTION OF MEGADIVERSITY

Rolf Georg Beutel, Institut für Spezielle Zoologie und Evolutionsforschung, FSU, Germany

A position of *Hexapoda* as a subgroup of *Pancrustacea* is confirmed by analyses of transcriptomes and also by morphological data. The cave-dwelling Remipedia (ca. 24 spp.), are likely the sistergroup of the megadiverse insects. The monophyly of *Hexapoda* is supported by molecular evidence, by the specific tagmosis, and by morphological apomorphies implied by the *Pancrustacea* concept (e.g. tracheal system). The interrelationships of the "basal" entognathous orders remain ambivalent. The monophyly of Insecta (=Ectognatha), Zygentoma, Dicondylia (Zygentoma + pterygote insects), and Pterygota is confirmed. Active flight with two pairs of wings was arguably the most important single innovation in hexapod evolution, providing an efficient escape mechanism, greatly improving the dispersal ability, and facilitating access to three-dimensional space, especially plant surfaces. The basal branching pattern in *Pterygota* remains ambiguous ("Palaeoptera problem"). The long disputed monophyly of *Polyneoptera* is confirmed by developmental features and transcriptomic data. The controversial *Zoraptera* are placed in this lineage, which is mostly characterized by plesiomorphic features. The interrelationships within *Polyneoptera* remain uncertain, even though a monophylum comprising *Xenonomia* (Grylloblattodea + Mantophasmatodea), *Eukinolabia* (Embioptera + Phasmatodea) and *Dictyoptera* (Mantodea + Blattodea incl. termites) is suggested by analyses of transcriptomic data, with the species-rich *Orthoptera* as its sister taxon. *Paraneoptera* (excl. *Zoraptera*) and *Holometabola* form a clade *Eumetabola*, even though this is weakly supported by morphological data. The monophyly of *Paraneoptera* remains ambiguous with respect to *Psocodea* (Psocoptera + Phthiraptera). *Thysanoptera* are the sistergroup of *Hemiptera*, which underwent an enormous radiation, likely triggered by mouthparts specialized on piercing and sucking fluids and the diversification of angiosperms in the late Mesozoic. The extremely diverse *Holometabola* comprise three large clades *Hymenoptera*, *Neuropteroidea* (Neuropterida + (Strepsiptera + Coleoptera), and *Mecopterida* (Amphiesmenoptera + Antliophora). The successful evolutionary interaction with angiosperm plants has triggered unparalleled diversifications, especially in the "Big4" *Hymenoptera*, *Coleoptera*, *Lepidoptera* and *Diptera*.

Keywords: Hexapoda, phylogeny, evolution, megadiversity

PL003

EDITING POPULATION GENETICS FOR VECTOR CONTROL

Andrea Crisanti, Department of Life Sciences, Imperial College London, UK

Mosquitoes species of the genus *Aedes* and *Anopheles* are responsible for transmitting severe and life threatening diseases including a number of viral encephalitis, Dengue yellow fever, Malaria and more recently Zika. A few Anopheles species are responsible for causing 200 million cases of malaria every year and the death of half a million children under the age of five in less developed regions of Africa. During the last twenty years a worldwide concerted effort based on the use of bed nets, insecticides and drug treatment has halved malaria morbidity and mortality. The implementation of these control measures necessitates about 10 billion per year mostly in the form of donations thus questioning the long-term sustainability of this approach and its suitability for eradicating the disease in the next 30-40 years. The vectorial capacity of a mosquito species to transmit malaria depends on genetically determined traits such as feeding behaviour, longevity, density and ability to support parasite development. Editing of the corresponding genes is anticipated to impair mosquito ability to transmit malaria. The recent development of CRISPR/CAS9 based gene drive technology has unlocked the possibility to selectively edit a mosquito population. Genetic modifications designed to either impair female fertility or interfere with mosquito ability to transmit the malaria parasite have been spread from few laboratory individual to large caged mosquito populations. These laboratory experiments have also supported mathematical modelling predicting how gene technology has the potential to eradicate malaria transmission in a span of few years from vast regions of Africa. Technical challenges in the development of a gene drive technology suitable for release include the development nuclease-resistant functional gene variant that would block the spreading of the drive as well as off target activity of the CAS9 nuclease that may generate undesirable mutations at other loci. We present here a number of solutions to overcome these problems.

Keywords: Gene drive, anopheles, malaria

PL004

MULTITROPHIC PLANT-INSECT-MICROBE INTERACTIONS

Marcel Dicke, Laboratory of Entomology, Wageningen University, The Netherlands

Plants are members of a complex community consisting of insects at several trophic levels, including herbivores (2nd trophic level), pollinators (2nd trophic level) and carnivores (3rd and higher levels). Plants play an important role in mediating interactions between individuals at different trophic levels. For instance, plants mediate competition between two herbivores through induced defences, or between two parasitoids that are each feeding in their own herbivorous host. Plants also mediate the attraction of carnivores to herbivore-infested plant tissues as well as the localization of parasitoids that feed within an herbivore by their hyperparasitoid enemies. At each trophic level, microorganisms are associated with macroorganisms. Recent evidence shows that microorganisms associated with plants, with herbivores and with carnivores can have important consequences for plant-mediated species interactions among insects a different trophic levels. I will highlight how this can influence population dynamics of macroorganisms.

Keywords: Microorganisms, herbivores, parasitoids, hyperparasitoids, plant-mediated interactions, ecology, community, population

PL005

HOW INSECTS MANAGE THEIR MICROBES

Angela E. Douglas, Insect Physiology and Toxicology, Cornell University, USA

All insects bear microorganisms, and most of these microorganisms are benign or beneficial – pathogens are the exception. A key determinant of how insects manage their menagerie of microbes is location. I will focus on insect interactions with microorganisms in two locations: in insect cells, focusing on intracellular bacteria in aphids and whiteflies, and in the insect gut, specifically the gut microbiota of drosophilid flies. The function and abundance of intracellular bacteria are regulated by metabolic and immunological properties of the insect host cell, as revealed by genome-based methods to investigate the patterns of nutrient exchange and key immunological factors. Turning to the gut microbiome of drosophilids, genome-based approaches have revealed multiple determinants of the composition and function of the gut microbiome, with evidence for effects of diet, host genotype and among-microbe interactions, as well as stochastic processes of passive dispersal and ecological drift. Despite the considerable variation among insect-microbial associations, as illustrated by my presentation, the one common feature is that microbes have a profound effect on insect traits and fitness. Insect-associated microbes are an integral part of any proximate or ultimate explanation of insect function.

Keywords: Aphid, bacteriocyte, drosophilid, gut microbes, microbiome, symbiosis, whitefly

PL006

INTEGRATING BIODIVERSITY SERVICES IN AGRICULTURE

Teja Tscharntke, Agroecology, University of Göttingen, Germany

Under the current scenario of rapid human population increase, combining efficient and productive agricultural land use with conserving biodiversity is a global challenge. A major argument for wildlife friendly farming and agroecological intensification is that crucial ecosystem services are provided by the ‘‘planned’’ and ‘‘associated’’ biodiversity. Loss of biological control can result in dramatic increases of pest densities, pollinator services affect a third of global human food supply, and inappropriate agricultural management can lead to environmental degradation. Hence, the true value of functional biodiversity on the farm is often inadequately acknowledged or understood, while conventional intensification tends to disrupt beneficial functions of biodiversity. Even during the last decades, high percentages of populations and species have gone extinct in human-dominated landscapes. This presentation is subdivided in three parts: Local management of biodiversity services, landscape design combining land sharing with land sparing, and at a global scale, adapted solutions for smallholders. Negative externalities of pesticide use, nitrogen losses as well as disappearing pollination, biocontrol and cultural services are discussed. Landscape structure is a key factor of local ecosystem processes, so we need to better understand how to design the configurational and compositional landscape heterogeneity. At a global scale, agriculture practiced under smallholder dominated landscapes, and not large-scale farming, is the backbone of global food security. In conclusion, linking agricultural intensification with biodiversity conservation and hunger reduction requires well-informed regional solutions at different spatial scales.

Keywords: Agroecology, conservation, landscape structure, habitat management, biocontrol, pollination

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Oral Communications

Session 1. Agricultural and Forest Entomology

Invasive Species Biology, Ecology and Management

CO001

CAN WE TRUST HISTORICAL DATA TO DISENTANGLE THE EVOLUTIONARY HISTORY OF INTRODUCTION? INPUT OF A GENETIC APPROACH TO COMPARE THE INVASION HISTORIES OF TWO SEED CHALCIDS (*MEGASTIGMUS SP.*) IN FRANCE

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Alain Roques, INRA, Orléans, France
Thomas Boivin, INRA, Avignon, France
Carole Kerdelhué, INRA, Montpellier, France

In recent decades, the number of introduced species has increased due to the development of human activities such as trades and transports. Insect species developing within fruits or seeds are particularly difficult to detect and may have a high invasive potential. The insect genus *Megastigmus* is a group of micro-hymenoptera that mostly develop in seeds. Several species of *Megastigmus* have been accidentally introduced in different parts of the world through the exchange of seeds carrying hidden Megastigmus larvae. Among these species, two were introduced in France a century apart: the Douglas fir seed Chalcid, *Megastigmus spermotrophus*, was introduced from North America to Europe at the end of XIXth century, and the cedar seed chalcid, *Megastigmus schimitscheki*, was observed for the first time in southeastern France in the early 1990's. To disentangle the evolutionary history of these introductions, we used two complementary approaches: (i) reviewing available historical data on the traceability of imported seed lots, and (ii) studying the patterns of genetic diversity of the invasive wasps in their native range compared to the invasive range. We combined mitochondrial sequences and nuclear microsatellites to characterize several populations from both the areas, and our results suggest that we can trust the historical data for the Douglas-fir seed chalcid (*i.e.* sourced from the North American coast) even if some genetic data indicate that the history in the introduced range is likely complex. On the other hand, genetic results demonstrate an unambiguously Cypriot origin for the cedar seed chalcid, although the available historical data strongly suggested that Turkey could be the most plausible source area. We conclude that historical data may provide incomplete, sparse or misleading information on invasion history, and genetic analyses are essential to reconstruct the whole history.

Keywords: Invasion, seed chalcid, historical data, phylogeography, population genetics

CO002

ALIEN FOREST INSECT INVASION TRENDS AND OUTLOOK

Eckehard Brockerhoff, Scion (New Zealand Forest Research Institute), New Zealand

Invasions of forest insects can be highly damaging and may have devastating consequences such as the extinction of host trees. After centuries of trade between Europe and North America, one would expect that the risk of invasions between these regions is diminishing, as the most successful invasive species are likely to have become established already, while the remainder are species that are less successful as invaders. Several recent studies have explored whether this is in fact the case, and whether biological invasions may eventually not even be a problem anymore. I will review some examples of data on forest insect invasions, the size of source pools (*i.e.*, the number of potentially invasive species), the fraction of species that have successfully invaded, and temporal patterns in the accumulation of these species. Bark and ambrosia beetles (Scolytinae) are an interesting group to address this question because they are well known and there are good records of successful invasions. Records of invasions of European Scolytinae that became established in North America suggest that there is indeed a decline over time in the rate of invasions, despite an increase in international trade, although many European species remain that could still invade in the future. Invasions of North American species in Europe have been less frequent, despite the larger size of the North American species pool. However, with the globalisation of international trade, new source pools are being accessed (*e.g.*, species native to parts of Asia and South America), and this is greatly increasing the number of potential invaders. Consequently, there are likely to be enough potential invaders to maintain a high rate of invasions of forest insects for several centuries into the future. These and other implications will be discussed, using mainly examples of invasions into Europe as well as European insects invading elsewhere.

Keywords: Insect invasions, outlook, prediction, invader depletion, Europe, North America, source pools

CO003

HOST'S MASTING DRIVES SPATIO-TEMPORAL PATTERNS OF PRE-DISPERSAL SEED PREDATION BY INVASIVE WASPS IN A NATURAL FOREST ECOSYSTEM

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In the context of trade globalization, forest ecosystems and tree plantations are increasingly subject to invasions by phytophagous insects. Seed insects are particularly prone to bioinvasions and are subsequently likely to affect plant populations through increased direct trophic interactions, which can result in more complex indirect effects on natural regeneration processes or intensively-managed seed orchards' productivity. Identifying both patterns and drivers of resource exploitation by invasive seed predators in their recipient ecosystem is a key step to forecast their impacts under both ecological and economic considerations. Within the general framework of herbivores' resource concentration/dilution hypotheses, we specifically addressed how spatio-temporal variations in host resource availability may drive spatio-temporal patterns of pre-dispersal seed predation (PDSP) by invasive wasps at the tree population scale. We used an ideal masting tree-wasp system (*Cedrus atlantica-Megastigmus*) surveyed in a 10-year longitudinal study under natural conditions in Southern France. Both fructification and PDSP patterns of individual trees were characterized over the period along with other individual tree and environmental characteristics. A mixed model was developed to assess how PDSP responded to spatio-temporal variations in these characteristics. We showed that spatial distribution of PDSP was significantly dependent on the interaction between annual fructification level and neighbouring host density: during mast seeding years wasps preferentially attacked trees that are at low density (resource dilution effect), while trees at high density concentrated wasp attacks during years of seed scarcity (resource concentration effect). This work highlights that host's masting may not only influence annual PDSP rates but also drive the spatial distribution of invasive seed specialists. We conclude that forecasting and managing spatial patterns of PDSP may require the consideration of both spatial and temporal dimensions of seed production at the tree population scale.

Keywords: Invasion, plant-insect interactions, fluctuating resource, resource concentration-dilution, Pinaceae, *Hymenoptera*

CO004

PHYSIOLOGICALLY-BASED DEMOGRAPHIC MODELLING APPROACH FOR INVASIVE ALIEN SPECIES: THEIR CONTRIBUTION FOR QUANTITATIVE PEST RISK ASSESSMENT

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Invasive alien species (IAS) represent an important threat for biodiversity and ecosystem services. They have also an important impact on plant, animal and human health and well-being. There is a growing need to develop methodologies for the quantitative assessment of risks posed by IAS to the environment as well as to plant, animal and human health, based on realistic scenarios of entry, establishment, spread and impact. Quantitative risk assessment can benefit from the contribution of population-based modelling approaches which are able to provide scenarios on the potential distribution and the dynamics of spread of IAS in newly invaded areas. We explore the use of a Physiologically-Based Demographic Model for the development of scenarios supporting quantitative risk assessment of invasive pests. Demographic models can describe spatio-temporal patterns of pests' population abundance which is considered the main driver influencing their impact. The physiologically-based approach accounts for non-linear relationships between environmental variables and biological responses at individual level. The invasion dynamics in Europe of the American grapevine leafhopper (*Scafoideus titanus*) and the Mediterranean fruit fly (*Ceratitis capitata*) are considered as case studies. For the two species we investigated the area of potential establishment and derived the spatial distribution through an index of their potential abundance in Europe. The possibility to mechanistically represent the influence of the environmental variables on demographic processes is used to explore the impact of climate change on distribution and abundance of the two species. Model outputs were compared with data showing actual presence of the specie in Europe. The modelling approach proposed provides the information necessary to perform quantitative risk assessment and might be suitable for (i) exploring consequences of different management scenarios for the control of IAS, (ii) comparing and prioritising IAS, (iii) supporting the prevention, early detection, rapid response and long-term control of IAS.

Keywords: *Ceratitis capitata*, *Scafoideus titanus*, quantitative risk assessment, Physiologically-Based Demographic Model, invasion dynamics, plant health

CO005

THE EMERALD ASH BORER AND THE THREAT TO NATIVE OLEACEAE: AN IMMINENT INVASION IN SOUTHERN EUROPE?

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An abundance of non-native, highly invasive insects in North America are threatening European countries, with devastating consequence. In particular, the introduction of the highly destructive emerald ash borer *Agrilus planipennis* (Colepotera: Buprestidae) appears imminent. Larvae feed on the phloem of ash trees (*Fraxinus spp.*) causing extensive canopy dieback. Attacked trees die rapidly. Clear signs of its presence are serpentine larval galleries visible under the bark, and D-shaped exit holes. Native to China, the beetle has killed millions ash trees in cities and forests of Eastern and Mid Western North America, but is currently present and expanding its range in European Russia. Furthermore, the recently reported utilization by the emerald ash borer of novel non-Fraxinus hosts in the family Oleaceae, and the spread of ash dieback caused by *Hymenoscyphus fraxineus* are adding concerns to the conservation of European Fraxinus species and other Oleaceae, including olive, *Olea spp.* We aim to: a) resume most recent knowledge on the pest's current distribution in Europe and its ecology in invaded areas, b) present natural distributions of native Oleaceae in the southern part of the continent, c) propose tools for forecasting invasion pathways and impact, and d) outline management strategies and predict challenges for mitigating the borer's proliferation through European urban and wildland forests.

Keywords: Invasive species, Europe, Forests, *Agrilus planipennis*, Fraxinus, Oleaceae

CO006

INSECT CHEMICAL COMMUNICATION DURING INVASIONS

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Phytophagous insects can locate host plants by detecting emitted volatile chemicals. To find mates and mount cooperative attacks against actively

defending host plants, insects may also produce sex pheromones or aggregation pheromones. Predators and other species of phytophagous insects can cue on any of these chemicals, leading to chemically-mediated interactions between phytophagous insects, host plants, and predators. For invading species lacking recent co evolution with species in the novel range, these chemical interactions may be weaker. Invading species may have difficulties locating hosts and be chemically inconspicuous to local predators. Navigating a novel semiochemical landscape will have a major impact on invasion success. Our research aims to predict the potential invasiveness of two bark beetle species, *Ips typographus* and *Dendroctonus rufipennis*, by studying semiochemical interactions with novel hosts and predators. Although we are unable to experimentally introduce the insects into novel ranges, we can take advantage of prior introductions of non-native tree species which put native bark beetles in contact with novel hosts. We are conducting host choice experiments in Europe and North America, respectively, to determine if *I. typographus* or *D. rufipennis* are attracted to the natural chemical odor of non-native host species, and whether they will infest and complete their life cycles within novel hosts. We are also conducting trapping experiments using synthetic pheromone blends to determine how native predators and other heterospecifics will respond to pheromone blends of *I. typographus* and *D. rufipennis* in invasion scenarios. Our results to date show that these two species may be pre-adapted to locating novel hosts but may also produce pheromone blends that are attractive to generalist predators in their potential invaded ranges.

Keywords: Invasion ecology, chemical ecology, bark beetles, pheromones, semiochemicals

CO007

NOVEL PHEROMONES FOR DETECTION AND MONITORING OF EXOTIC INVASIVE SPECIES, AND ENDANGERED NATIVE SPECIES OF CERAMBYCID BEETLES

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The past 15 years has seen a huge paradigm shift in our understanding of the use of attractant pheromones by the large beetle family Cerambycidae. Historically, it was hypothesized that volatile pheromones were rare and of little importance in the biology of cerambycids. However, there is now abundant evidence that, as with many other insect families, pheromones are crucial mediators of reproductive behaviors for most if not all cerambycid species, with pheromones now identified from several hundred species. Initially, it appeared that pheromone structures were highly conserved within related species, but recent work has shown that pheromones vary along a continuum, from highly conserved and used by many species on several continents, to compounds used only by a few closely related species, to compounds which may be species-specific. The pheromone compounds are also very diverse chemically, arising from a variety of biosynthetic pathways. For practical purposes, these pheromones can be readily exploited for detection and sampling of cerambycids, particularly when species are cryptic, or present in low densities and difficult to detect. Examples will be presented of cerambycid pheromones for monitoring known invasive species, such as several species in the genera *Callidiellum* and *Monochamus*, *Trichoferus campestris*, and the notorious *Aromia bungii* which has recently invaded Italy. Conversely, examples will be presented of the potential use of pheromones for monitoring endangered species, such as the red-listed *Rosalia alpina* in Europe, and *Desmocerus californicus* in North America.

Keywords: Cerambycidae, pheromone, surveillance, monitoring, invasive species, endangered species

CO008

FEEDING ECOLOGY OF THE JAPANESE BEETLE (*POPILLIA JAPONICA*), A MAJOR INVASIVE THREAT TO EUROPEAN AGRICULTURE

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The Japanese beetle (JB), one of the worst invasive pests in North America, was first detected in continental Europe, in northern Italy, in 2014. Because of its broad host range (>300 plant species) and propensity for its grubs and adults to be transported in commerce, JB is poised to become a major pest of agriculture, floriculture, nurseries, gardens, and turfgrass throughout much of temperate Europe. This talk summarizes >35 years' research on JB feeding ecology and applications to pest management. JB are mildly attracted to constitutive volatiles from many types of plants regardless of their suitability as hosts. Plant secondary chemistry drives acceptance/rejection at leaf surface. Likes sharks to a blood trail, additional JB are attracted to complex blends of feeding-induced plant volatiles resulting in aggregation and rapid defoliation usually beginning in upper sun-exposed portions of the plant. Most mating occurs on host plants, so induced odors also serve as an indicator of others feeding on a high-quality host. JB also bite in to ripe fruits, damaging them directly and contaminating them with yeasts that elicit fermentation odors that attract secondary fruit-feeding pests. Agricultural applications of this research including use volatile lures and host plant resistance in JB management will be briefly discussed.

Keywords: Japanese beetle, *Popillia japonica*, invasive pest, biosecurity, insect-plant relationships, induced plant volatiles

CO009

NEWLY-ARRIVING INSECT INVADERS IN EUROPE: NOT THE ONES WE PREVIOUSLY EXPECTED, AND SPREADING MUCH FASTER THAN BEFORE

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Globalization is resulting in an exponential increase in the establishment of non-native insects in Europe, with several species having substantial ecological and economic impacts. No saturation in the arrival of such species is observed, and a significant part of the new arrivals correspond to species that have never been introduced before outside their native range. Phytophagous species, especially those related to woody plants, largely dominate the recent introductions. Moreover, once established the recently-arrived non-native species appear to spread faster than those having arrived during the last centuries. The total area invaded by 1171 insect species for which the date of first record in Europe is known, was used to estimate their current range radius. The initial rate of radial spread was compared among different groups of insects for all years (1800–2014) and for a subset of more recent decades (1950–2014). Decreasing spread rates over residence time were observed in phytophagous species associated with herbaceous plants and crops but much less in those related to woody plants. Initial spread rate was significantly greater for species detected after 1990, roughly 3-4 times higher than for species that arrived earlier. It is hypothesized that the political changes in Europe following the collapse of the Iron Curtain in 1989, and the further dismantling of Customs checkpoints within an enlarged European Union (EU) have facilitated the faster spread of alien insect species. Also, the number of species first recorded in the Eastern Bloc of the politically-divided Europe before 1989 was lower than for the rest of Europe. A detailed analysis of several recent invaders associated to woody plants indicated a dominant role of long-distance translocations across Europe related to human activities, especially with the plant trade, in determining rates of spread. The additional role of global warming in favouring expansion will be discussed.

Keywords: Insect, invasion, plants, trade, spread

New Approaches for the Management of Agricultural and Forest Pests

CO010

MULTI-TACTIC STRATEGIES FOR THE MANAGEMENT OF PLANT FEEDING MITES

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Pesticide spray is the prevailing method for insect and mite control in agriculture. The continuous reliance on pesticides can bring substantial environmental liabilities of off-target drift, chemical residues, pest resistance, trophic network alterations, control cost increase, etc. *Tetranychidae* (spider mites) and *Eriophyoidea* (gall-making mites) are the most economical important plant mite feeders. They are associated to annual as well as perennial plants, such as crops, ornamentals and weed species within *Pteridophyte* (ferns), *Gymnosperm* and *Angiosperm* taxa. Most Tetranychid species are polyphagous, provided with stylet-like chelicerae and include species highly harmful to economic plants. Eriophyoid mites have highly specialized mouthparts and most of species have a high degree of host specificity with the neat prevailing of the strict monophagy. Moreover, a number of eriophyoid species transmit plant viruses. Integrated Mite Management approach to control plant feeding mites (spider and gall-making mites) are briefly reviewed with particular attention to the most recent tools in the view of multi-tactic strategies. This is the case of host-plant resistance, augmentative and inundative biological control (taking into account also acaricide-resistance predatory mites), biorational tools, non-transgenic methods and genome editing. The impact of transgenic plants on plant and soil inhabiting mites is also commented.

Keywords: Acari, spider mites, gall-making mites, control

CO011

MERGING DNA-MATABARCODING AND ECOLOGICAL NETWORK ANALYSIS TO UNDERSTAND AND BUILD RESILIENT FARMLAND ECOSYSTEMS

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There have been significant recent advances in both mathematical and molecular approaches in ecology, offering unprecedented opportunities to describe and understand ecosystem functioning. Ecological networks describe interactions between species, the underlying structure of communities and the function and stability of ecosystems. Using ecological networks provides the ability to assess the robustness of complex ecological communities to species loss as well as a novel way of guiding restoration. However, empirically quantifying the direct and indirect interactions between entire communities, and across multiple trophic levels, remains a significant challenge. Concomitantly, advances in DNA sequencing technologies are resolving previously intractable questions in functional and taxonomic biodiversity and provide enormous potential to determine hitherto difficult to observe species-interactions. Combining DNA metabarcoding approaches with ecological network analysis presents important new opportunities for understanding large-scale ecological and evolutionary processes as well as providing powerful tools for building ecosystems that are resilient to environmental change. We provide examples of how molecular approaches have enhanced the ability of ecologists to study species-interaction networks to date and propose a novel 'nested tagging' metabarcoding approach for the rapid construction and analysis of large, phylogenetically structured species-interaction networks. Using farmland species-interaction networks as an example, we provide a conceptual framework for constructing and analysing 'networks of ecological networks' using metabarcoding. We show how qualitative and quantitative measures of network robustness can be used to determine the consequences of species loss within farmland and farmland habitat loss within wider landscapes. By determining which species and habitats are disproportionately important to network integrity, we propose new directions for building robustness into agro-ecosystems of the future.

Keywords: Food-webs, pollination, natural pest control, EcoStack

CO012

EFFICIENCY OF INDUCED PLANT DEFENSES AGAINST INSECT FEEDING DAMAGES AT DIFFERENT RISK LEVELS

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Methyl jasmonate (MeJA) treatment is a promising method to protect planted conifer seedlings against damage caused by the pine weevil (Hylobius abietis). By applying an exogenous plant defense elicitor – such as MeJA – defenses can be induced before an attack by herbivores and damage levels can thus be reduced. Adult pine weevils feeding on the bark of unprotected young conifers may cause plant mortality rates of up to about 90%. Hence the pine weevil is one of the most important forest pest insects in Europe. Using MeJA to protect seedlings has been evaluated in a previous field experiment with good effect. Pine weevils are, however, able to feed on MeJA-treated seedlings, especially when they have no other option (*e.g.* under no-choice laboratory conditions). Consequently, it is possible that pine weevils feed extensively on MeJA-treated seedlings in the field when damage risk levels are high. We therefore assessed the efficiency of MeJA treatments to protect Norway spruce (*Picea abies*) seedlings exposed to varying levels of pine weevil pressure. This was achieved by planting seedlings on different clear-cuts and in different soil types. Preliminary analysis shows that the MeJA-induced defense systems reduced damage but the protective effect was most pronounced at medium risk levels. At the highest risk levels the protective effect was not sufficient to reduce the seedling mortality to acceptable levels. A damage-reducing effect of MeJA treatment was obtained even with the lowest concentration tested (5 mM). Higher MeJA concentrations (10 mM and 15 mM) did not considerably improve the protective effect. The results indicate that MeJA treatment may become a tool for protection of conifer seedlings when the risk of pine weevil damage is moderate or when used in combination with some other damage reducing measure.

Keywords: Methyl jasmonate, Norway spruce, pine weevil, seedling protection

CO013

A DNA BARCODE APPROACH TO DEFINE TROPHIC INTERACTIONS BETWEEN NATIVE AND EXOTIC STINKBUGS AND THEIR PARASITOIDS

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Establishment of the brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), outside of its native range has generated interest in defining host-parasitoid associations between pentatomids and their parasitoids. Baseline information on parasitoid-pentatomid associations in natural habitats is lacking, particularly for non-pest species, and species-level identification of these associations can be tenuous using conventional rearing and dissection techniques. To address this, naturally occurring pentatomid eggs were collected in areas where *H. halys* has established in Canada. Eggs were analysed using a nested DNA barcoding approach to define species-level trophic interactions. Species-level identification was possible for >90% of egg masses. Eleven pentatomid and five scelionid species were identified, and trophic links between them were established. Approximately 70% of egg masses were parasitized; parasitism and parasitoid species composition were described for each pentatomid species. *Telenomus podisi* Ashmead was the dominant parasitoid and was detected in all host species. *Trissolcus euschisti* Ashmead was detected in the majority of host species, but was significantly more prevalent in *Chinavia hilaris* (Say). *Trissolcus brochymenae* Ashmead and *Tr. thyantae* Ashmead were only recorded sporadically. Parasitism of *H. halys* was 55%, and consisted of three species (*Te. podisi*, *Tr. euschisti*, and *Tr. thyantae*). Although these species are not able to develop in fresh eggs of *H. halys*, molecular tools demonstrate that parasitoids attempt to exploit this host under natural field conditions. Extensive knowledge of viable and nonviable host-parasitoid associations in the field can help evaluate non-target impacts associated with the establishment of exotic pests and biological control agents.

Keywords: Biological control, trophic interactions, host-parasitoid associations; brown marmorated stink bug; parasitoids

CO014

HIGH PROPORTION OF GRANIVORY IN CARABIDS EMPHASIZE IMPORTANCE OF WEED SEED PREDATION IN ARABLE LAND

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Trophic interactions between species provide ecosystem services such as weed seed predation in agroecosystems. Carabid beetles are an abundant and diverse group in arable land across Europe and are presumed to play a key role in regulating weed seeds. However, trophic functional groups of the different carabid species are not well defined yet. Most findings are based on laboratory feeding experiments or correlative analysis, making it difficult to evaluate the actual role of carabid beetles as seed predators in agroecosystems. Here, we used diagnostic polymerase chain reaction (PCR) to analyze the diet of different carabid species. Beetles were collected in organic wheat fields in three different regions in France and Austria during two collection periods, in May/June and July/August 2016. Regurgitates of the most abundant carabid species were molecularly analyzed (n=2359) using a general plant primer for trnL, a chloroplast gene, group-specific primers for earthworms and collembolans as well as three species-specific primers for aphids. The 22 selected species comprise carabids of 2.5-3.6 mm in size (*Bembidion quadrimaculatum*) for the smallest up to 25.0-35.0 mm (*Carabus scheidleri*) for the largest ones. Our results indicate that the proportion of granivory in carabid species diet is much higher than previously assumed. This includes also species, which had been described as predominantly carnivorous so far, suggesting that previously trophic functional groups of carabid beetles needs to be rethought. Moreover, the overall high share of seeds in the diet of the majority of carabids emphasizes their importance in biocontrol for weeds, which might significantly contribute in achieving international goals of reducing herbicide applications without compromising crop yields.

Keywords: Carabidae, granivory, trnL, trophic interactions, seed predation

CO015

USING POPULATION GENETICS AND GENOMIC APPROACHES TO DECIPHER THE RECENT HISTORY OF FOREST PEST INSECTS AND IMPROVE MANAGEMENT STRATEGIES

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In the last decades, population genetics has proved to be a powerful tool to study differentiation and divergence of species at different spatial and temporal scales. Classical molecular markers, mostly mitochondrial sequences and microsatellite loci, were successfully used to characterize the natural structure of populations, to identify the environmental factors favouring or impeding gene flow, and to understand the driving forces of genetic differentiation. In the context of current global changes, population genetics was also used to disentangle the demographic processes during invasions and expansions and to characterize dispersion patterns. These approaches have often highlighted the complexity and the sometimes counterintuitive nature of the on-going scenarios. The development of new model-based methods, such as approximate Bayesian computation (ABC), allows quantitative inferences and the explicit comparison of alternative scenarios. The recent advent of Next Generation Sequencing (NGS) technologies has revolutionized the field of population genetics, and now allows the development of genome-wide approaches even in non-model organisms. These major improvements provide most powerful tools to analyse the evolution of both neutral and adaptive genetic diversity. Taking the pine processionary moth as a case example, we will illustrate how population genetics and genomics can be used in forest entomology and bring valuable answers regarding invasion and expansion processes. In particular, we could identify signs of both diffusive dispersal and long-range movements during the expansion of this species in France, which suggests man-aided movements possibly linked to host plant trade. We will also show how genomic markers (RAD-seq) were developed and allowed to deeply explore a case of recent allochronic differentiation in Portugal, which resulted in the occurrence of a phenologically-shifted and highly divergent population for which specific management strategies must be applied.

Keywords: Population genetics, entomology, expansion, differentiation, allochrony

CO016

ECOLOGICAL AND EVOLUTIONARY DRIVERS OF VARIATION IN PLANT RESISTANCE AGAINST HERBIVORES

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In most food webs, insect herbivores are one of the major conduits of energy flow between autotrophic plants and the higher trophic levels. Thus, it is not surprising that insect herbivory has led to the evolution of remarkably diverse and potent plant defences. Natural selection imposed by insect herbivores appears to have resulted in the evolution of sandpapery leaves, digestion inhibitors, toxins as well as information chemicals that can be exploited by natural enemies to reduce herbivore fitness. Over the last half century, complementary theories and hypotheses have been developed to try to explain the extraordinary variation in plant defensive strategies, and, thanks to interdisciplinary interaction between ecologist, behaviourists, physiologist, and chemists, it has given rise to the body of work, collectively known as "plant defence theory". Nowadays, advances in community phylogenetic and metabolomic analysis are the key components for refining plant defence theories at a novel frontier. I will show examples of how regulation of plant defence strategies can be affected across the whole hierarchical organization of life, spanning from ecosystems to organisms and to genes. I will thus argue that only a holistic approach incorporating large-scale ecological gradients will enable us to fully grasp ecology and evolution of plant defences against herbivores.

CO017

FROM LAB TO REGISTRATION: AN ATTRACT & KILL CONTROL STRATEGY FOR WIREWORMS IN POTATO PRODUCTION SYSTEMS

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Wireworms are currently regarded a serious pest problem in conventional and organic potato production systems in Europe. Control options targeting wireworms are limited, due to the phasing out of effective insecticides in Europe or are ineffective. We developed an innovative control strategy using a formulation which combines the application of a wireworm attractive compound with a specific killing agent (entomopathogenic fungus). Wireworms use carbon dioxide gradients to locate roots of host plants. We made use of this orientation cue by developing a dry capsule system which re-hydrates upon application into the soil producing CO_2 (*i.e.* attract component). Simultaneously an isolate of *Metarhizium brunneum* inside the capsules (*i.e.* kill component) grows out of the capsules and sporulates on the surface. When coming into contact with these A&K capsules, wireworms are infected and will be killed. To make this strategy work under field conditions, the capsules need to build up CO_2 gradients significantly higher than the background CO_2 concentrations in the soil for at least several weeks. Moreover, the entomopathogenic fungal isolate needs to target the different wireworm species typically found together in a potato field. This capsule type has now been registered in Germany and Austria (ATTRACAP®), based on clause 53 of EU regulation 1107/2009 and has been proven effective in farmer fields in 2016 and 17 with an average control efficacy above 60%. The use of the product is somewhat constraint by abiotic and biotic parameters, which will be addressed in detail during ongoing field tests and capsule adjustments. Moreover, although the final registration of ATTRACAP® is initiated, several restrictions, such as data acquisitions, and additional field evaluations, will delay final registration. The implementation roadmap for this strategy will be discussed in detail.

Keywords: *Metarhizium brunneum*, *Agriotes spp*, innovative control strategy, sustainable potato production

CO018

CLIMATE CHANGE AND BIOLOGICAL CONTROL - TOO HOT FOR SOME

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Climate change imperils global food security by compromising agricultural production. The prevailing effects of climate change have resulted in organismal range shifts and population changes, and they are increasingly considered to pose a risk to species extinctions. The biological control of pests of food crops is a key ecosystem service that underpins sustainable approaches to their management, providing significant fiscal and environmental benefits. The impacts of climate change on host-parasitoid interactions, whether natural enemies deliberately introduced into new regions, so called classical biological control, or whether the agents are indigenous and biological control is being supported by conservation practices, will be modulated by direct effects on the organisms involved (*e.g.* through effects on physiology and metabolism), the responses of those organisms and subsequent tri-trophic interactions. Parasitoids, which represent the third trophic level, are likely to be significantly affected by climate induced perturbations to these systems and understanding what these effects might be is of critical importance. The relative thermal requirements and tolerances of hymenopteran parasitoids and their hosts were investigated based on published data. The optimal temperature (Topt) for development of parasitoids was significantly lower than that for their hosts. Given the limited plasticity of insect responses to high temperatures and the proximity of Topt to critical thermal maxima, this suggests that host-parasitoid interactions could be negatively affected by increasing global temperatures. A modelling study of the interactions between the diamondback moth and its parasitoid *Diadegma semiclausum* in Australia indicated that predicted temperature increases will have a greater negative impact on the distribution of the parasitoid than on its host and that they could lead to its exclusion from some agricultural regions where it is currently important. We illustrate other possible outcomes using a simple modelling approach.

Impacts of Climate Change on Agricultural and Forest Pests

CO019

TEMPERATURE-DEPENDENT PHENOLOGY AND CLIMATE CHANGE MODEL OF CASSAVA-COLONISING POPULATIONS OF *BEMISIA TABACI*

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The whitefly, *Bemisia tabaci*, transmits viruses that are the greatest constraint to the production of Africa's most important food security crop – cassava. We aimed to model the impact of climate change on cassava-colonising *B. tabaci* and assess the changing risks posed by this global pest. We studied the life history of an endemic African population of *B. tabaci* (SSA1-SG3) at six constant temperatures (16, 20, 24, 28, 32, 36°C) in controlled environment chambers, and for 10 generations in the field. Our results show that the whiteflies preferred temperatures from 20°C to 28°C for oviposition, and that total fecundity (117.5 eggs per female) was highest at 20°C. Similarly, *B. tabaci* adult females lived longest (19.7 days) at 24°C compared to other temperatures, and longevity was least at 36°C (8.5 days). Immature developmental time decreased with temperature up to 28°C. It was slowest at 16°C (59.3 days), and fastest at 28°C (16.3 days). Eggs did not develop successfully to adults at 36°C and this temperature was therefore considered lethal. In the field, development duration varied from 18.0 days during one of the hottest months, to 25.0 days during the coolest month. Mean immature survival peaked at 24°C (62.5%), while least survival (14.9%) was observed at 16°C. Natural enemies had a significant influence on immature survival rates. Several models describing temperature-dependence of insects were fitted to these life history data, and an overall phenology model was developed for this pest using Insect Life Cycle Modelling (ILCYM®) software. The established phenology model predicted maximum population growth between 22 and 24°C. A comparison of *B. tabaci* SSA1-SG3 to *B. tabaci* MEAM 1 and MED suggests that both *B. tabaci* MEAM 1 and MED are probably more fit compared to *B. tabaci* SSA1-SG3.

Keywords: *B. tabaci*, phenology modelling, climate change, cassava, Africa

CO020

INSECT DEFOLIATORS AND CLIMATE CHANGE: EFFECTS ON TOP-DOWN AND BOTTOM-UP PROCESSES

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Insects have responded to climate change. For instance, the distributions of many species have moved northwards and to higher altitudes. For some pest species, like the spruce bark beetle, there are indications of increasing damage directly related to increasing temperatures. However, for a large number of species such a direct link as not been established and for some species levels of and fluctuations in herbivory might have declined. As the level of and fluctuations in damage is related to the density of the insect, we need to understand mechanisms underlying population dynamics to predict the likelihood of damage under climate change. For defoliating insects, like many lepidopterans and sawflies, bottom-up mechanisms acting via the quality of the host plant and bottom-up mechanisms acting via the natural enemies of the herbivores are important for their population dynamics. As both the host plants and the natural enemies, which to a large part are other insects, as well as the defoliators are affected by and will most likely respond differently to changes in climate, it becomes crucial to investigate how all three trophic levels respond to *e.g.* increasing temperature or changes in precipitation. We will present results from a literature review where we summarize the effect of bottom-up and top-down mechanisms on traits related to defoliator population dynamics, *i.e.* traits like survival and weight (linked to fecundity in many species). One aim is to elucidate whether top-down processes are more sensitive to climate change than bottom-up processes, as has been predicted by some authors. We link the results of bottom-up and top-down processes responses to observed patterns in damage and try to find differences and similarities between species and groups of defoliators.

Keywords: Insect defoliators, population dynamics, damage, bottom-up, top-down

CO021

TOO HOT TO HANDLE? INTERACTION OF AGRICULTURAL PESTS AND CLIMATE CHANGE

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The agricultural sector is directly affected by changes in air temperature, precipitation and climate extremes but also contributes to climate change by emissions of anthropogenic greenhouse gases and land use changes. To assess the expected impacts for Luxembourg, we analyzed regional climat change projections based on the ENSEMBLES and EURO-CORDEX data-sets. A multi model ensemble of transient projections until year 2100, driven by the different emission scenarios like A1B, RCP2.6 and RCP8.5, were used. The daily data of mean air temperature and precipitation for a 3x3 grid box – centred over Luxembourg – were calculated and used as driver for the impact models (*e.g.* pest abundance and migration). Because Regional Climate Model (RCM) outputs were systematically biased in comparison to observations, a bias correction approach was used to correct air temperature and precipitation data. Based on long-term, multi-sited field observations, the abundance and migration time of different insects pests in oilseed rape (*Brassica napus L.*) were observed and linked with corresponding meteorological data-sets, to develop models for climate change impact studies. So far, impact studies for the rape stem weevil (*Ceutorhynchus napi*), cabbage stem weevil (*Ceutorhynchus pallidactylus*), pollen beetle (*Brassicogethes aeneus*) as well as for the pod midge (*Dasineura brassicae*) were dirived. In general, a shifting of migration to an earlier onset but also a prolongation of migration periods can be expected under climate change conditions. For *C. pallidactylus*, an additional model was developed to forecast if the economical threshold for brassicaceous host plants will be breached and - in consequence - chemical treatment would be necessary. This approach is also used to calculate the pest status of *C. pallidactylus* under future environmental conditions. All these forecast models are integrated in an holistic decision support system that is used in Luxembourg for advising farmers in plant protection issues.

Keywords: Climate change, CORDEX, emission scenario, insect pests, oilseed rape, pests

CO022

DEALING WITH HIGH TEMPERATURES: THE APHID *ACYRTHOSIPHON PISUM* EXHIBITS A GREATER SURVIVAL AFTER A HEAT SHOCK WHEN PARASITIZED BY THE WASP *APHIDIUS ERVI*

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An increase in the frequency of extreme thermal events is expected as a result of global climate change. This increase, together with the mean global warming, may impact on crops productivity by affecting, among others, biotic interactions between agricultural pests and their natural enemies. In many host-parasitoid interactions, both symbionts can be affected by extreme temperatures, and any effect on the host will have consequences on the inhabiting parasitoid. In this study we report how the parasitoid *Aphidius ervi* copes with the exposure to a heat shock of its host species, the pea aphid *Acyrthosiphon pisum.* We recorded a greater survival rate of parasitized aphids compared with unparasitized ones, after exposure to a high temperature (39°C for 30 min). After the heat shock, the survival of unparasitized aphids decreases according to their age at the heat shock treatment, suggesting a different adaptation of the aphid life stage to the different microclimatic conditions they experience. Survival of parasitized aphids does not change according to the time of the heat shock treatment, but it is always significantly higher compared with the unparasitized ones. The *A. pisum-A. ervi is*, to our knowledge, the first system in which a greater survival after a heat shock linked to a metazoan parasite has been described. The possible explanations, as well as the possible adaptive nature of the observed phenomenon are discussed.

Keywords: Host-parasitoid interactions, thermal stress, climate change

CO023

IS HOTTER BETTER FOR A MEDITERRANEAN PEST? A CASE STUDY WITH AN EXPERIMENTAL IMITATION OF GLOBAL WARMING BASED ON CLIMATE MODELLING

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Predicting the species and ecosystems responses to climate change has become one of the most challenging issues in ecology since a few decades. This requires to track the variation in individual performance following exposition to rising temperatures. The experimental procedure usually consists of applying a given increment to current thermal conditions, according to the expected increase in global mean temperature. Here we followed a realistic experimental design by assessing the variation in performance of an ectotherm experiencing current or future thermal regimes, simulated at a local scale by means of climate modelling. The climate models we used highlighted two facets of global warming in Burgundy, one of the main wine growing region in France: an increase in mean temperature (+5.3°C) and an increase in the amplitude of daily thermal fluctuations (+0.6°C). We then assessed the effects of mean temperature and thermal fluctuations on the global performance of the major pest evolving in vineyard, the European grapevine moth *Lobesia botrana*. We found that immune traits responded to the simple effects of mean temperature and fluctuations: immune function was reduced in future warmer conditions and in fluctuating regimes compared with constant ones. Development time and energetic reserves were rather impacted by an interactive effect between these two factors: thermal fluctuations associated with a higher mean temperature slowed down development and depleted energetic reserves. Such an interactive effect might be explained by the fact that maximum daily temperature is expected to increase more steeply than minimum temperature according to the climate models' projections. Hence, the insects experiencing future fluctuating conditions might have been regularly exposed to excessively high temperatures, resulting in a chronic stress depressing performance. In the context of global warming, our results show that several aspects of performance might respond differently to the alteration of mean temperature and fluctuations likely to occur in coming decades.

Keywords: Global warming, climate models, insect pest, vineyard, immunity, energetic reserves

CO024

THE RESPONSES OF 31 MAJOR GLOBAL INSECT PESTS TO ONGOING CLIMATE CHANGE - UNEXPECTED RESPONSES AND KNOWLEDGE GAPS

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Phytophagous insect pests strongly affect the productivity and profitability of agriculture and forestry. Despite the well-known sensitivity of insects to *e.g.* temperature their potential responses to ongoing climate change remain unclear. Here we review documented climate change responses of 31 of the globally most severe insect pests of forests and agriculture with a focus on species for which long-term, high-quality data are available. Most of the selected species show at least one response affecting severity, including changes in geographic range, population dynamics, life-history traits, and/or trophic interactions. Agricultural pests show strikingly more diverse and generally weaker responses to climate change than forestry pests, possibly due to several ecological and evolutionary factors. However, agricultural pests, historically restricted to their cultivated crop seem to increase in detrimental ecological impact to a greater degree than do forestry pests. Unexpectedly, 59% of the species show responses of reduced potential impacts as pests already under current climate change. Such reduction in impact is also supported by a thermal tolerance analysis that shows a general reduction in warming tolerance under both current climate and future projections. The documented variability in responses among the pest species indicates that efforts to mitigate undesirable climate change effects must target specific species. Several recent studies have called for information on biological mechanisms such as demography, dispersal, evolution, and trophic interactions when predicting and understanding climate change responses. Here we review such information, as well as identify knowledge gaps, for 31 important pest species and argue that the results may represent also other organism groups.

CO025

LINKING BARK BEETLE ATTACK OF NORWAY SPRUCE TO PERIODS AND INTENSITY OF DROUGHT

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Linking bark beetle attack of Norway spruce to periods and intensity of drought. The observed increase in the frequency and severity with which bark beetle disturbances occur in conifer forests worldwide is to a large part driven by weather events. Drought episodes are widely considered to stress host conifer trees and render them highly susceptible to scolytid attack. An *in situ* drought manipulation experiment (Rosalia Roof Project) showed that spring and early summer tree transpiration deficits, *i.e.* the reduction from a potential transpiration caused by soil water limitation particularly predispose Norway spruce stands to attacks by the Eurasian spruce bark beetle *Ips typographus*. Based upon the empirical outcomes of this study, we regard the occurrence of transpiration deficits as a meaningful index, and we thus integrated a simple water balance module (Tdef) in *I. typographus* hazard assessment. The novel tool Phenips-Tdef was designed to indicate the stress and thus susceptibility status of Norway spruce stands in relation to actual swarming times of bark beetles. We practically evaluated Phenips-Tdef for Norway spruce dominated forest stands of a large Austrian Forest Enterprise on sites representing a variety of climatic and soil conditions. For each site, we retrospectively simulated bark beetle development by the phenological model Phenips. To gain information on the seasonal incidence and intensity of drought over a period of several consecutive years, we applied the forest water balance module Tdef, and compared the outcomes with timber volumes harvested by the forest enterprise due to bark beetle infestation. The model is capable of real time, stand level reporting of periods of *I. typographus* swarming and generation development as well as of periods of drought stress and thus increased attack susceptibility of Norway spruce stands. Phenips-Tdef is thus well suited to support forest managers in prioritising and scheduling pest control and prophylactic measures.

Keywords: Drought, Transpiration deficit, Norway spruce, *Ips typographus*, Bark beetle risk assessment, Monitoring tool Phenips-Tdef

CO026

DENDROECOLOGICAL ANALYSIS OF THE ALTITUDINAL SHIFT OF LARCH BUDMOTH OUTBREAKS WITH CLIMATE CHANGE IN THE FRENCH ALPS

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Larvae of larch budmoth (*Zeiraphera griseana*; LBM) periodically defoliate alpine stands of European larch (*Larix decidua*) during massive outbreaks occurring at 8-10 year intervals, with an optimum at 1800 m altitude. Severe defoliation decreases the trees' photosynthetic potential and growth, especially resulting into narrower ring width. We investigated annual-ring time series in order to reconstruct the history of LBM outbreaks between 1963 and 2007 along a larch altitudinal gradient ranging from 1350 to 2300 m, in the Briançon area of the Southeastern French Alps,
A comparison of the annual-ring time series measured on the larch host trees with the annual variations in LBM population density estimated by branch samplings in a stand located a few kilometers apart revealed an excellent temporal coincidence between the occurrence of LBM outbreaks and the width of the rings. LBM outbreaks not only affected ring width but also other annual-ring variables, including traits related with sap conduction. Finally, the study of the annual-ring time series along the altitudinal gradient showed a recent altitudinal shift of LBM outbreaks from medium towards higher elevations, over 2000 m. We suggest that this altitudinal shift is a consequence of the variable effect of global warming at the different elevations. Longer and warmer summer improves larch life conditions at the top of the gradient while increased summer drought may have a harmful effect at the bottom. Winter warming is also expected to affect differently the timing of LBM egg hatch and that of larch bud flush, larvae being at present susceptible to emerge whereas no needles are available as food at the former optimal altitude. A better synchronization between larch and LBM may exist at higher elevations. Whether the larch ecosystem will be able to adapt at all points of its altitudinal distribution is still an open question.

Keywords: Larch, *Larix decidua*, larch budmoth, *Zeiraphera griseana*, ring width, altitudinal gradient

CO027

HOW DOES TEMPERATURE INDIRECTLY INFLUENCE INSECT HERBIVORES VIA THEIR HOST PLANTS?

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Previous temperature experiments with herbivores (pea aphid, *Acyrthosiphon pisum*) and host plants (fava, *Vicia faba*) exposed to an array of long time constant temperatures showed treatment effects on aphid population growth and plant development. Direct effects of temperature to aphids were expected, but how does temperature indirectly influence aphids via their host plants? We first planted fava seeds at different times in each temperature (16°C, 24°C, 32°C) so plants would be the same size at infestation. Plants were then moved to a common environment and infested with aphids. Any differences in aphid fecundity would be due to the plants history at different temperatures. However, we found no treatment effects on aphids suggesting that after standardizing for plant development, plant history did not play a substantial role in temperature effects. Our second, complementary experiment used plants that were planted at the same time and kept in a common environment (22°C). Some plants were then randomly assigned to temperature treatments (16°C, 24°C, 32°C) and infested with individual aphids. Half those plants stayed in the treatment with their aphids and the other half were replaced each day for five days with plants from the common environment. Aphid fecundity varied with temperature, but replacing plants had no effect suggesting no difference in aphid performance on plants kept in experimental temperatures over five days *versus* only one. Together, these results suggest that in our lab experiments differential plant development over long time periods is the primary mechanism for indirect effects of temperature on aphids.

Keywords: Temperature, pea aphid, *Acyrthosiphon pisum*, indirect effects, host plants

Monitoring and Surveillance of Insect Pests

CO028

THE OPTIMIZED UTILIZATION OF ROCKET TRAPS, AGGREGATION PHEROMONES AND ATTRACTANTS FOR MASS TRAPPING OF HEMIPTERAN BUGS IN DIFFERENT AGRO-ECOSYSTEMS

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Hemipterans especially, Brown-marmorated stink bug (BMSB), *Halyomorpha halysl*, and bean bug (BB), *Riptortus pedestris* Thunberg are major insect pests of several leguminous, cereals and fruit crops in Korea. Recently, species specific aggregation pheromones (APs) are being used for monitoring and mass trapping of two different stink bugs. We investigated the attractiveness of different Rocket traps (manufacturer: b2m Co. Ltd., Korea) baited with species specific APs along with food combinations of soybean seed and dried anchovy in different crop fields. As a result, the peak occurrence of over-wintered and newly emerged BB population was observed in early April and late August to early September, respectively. However, higher population of over-wintered and newly emerged adults was observed in middle May and early October, respectively. Generally, BMSB and BB were found to more active at 14:00 to 16:00 PM. BMSB and BB were significantly higher to the Rocket trap than that of the pyramid trap. Both bug species were also highly attracted to the Rocket trap with black colored wing and landing board than that of other colored rocket traps, and their attraction was further increased with multiple combinations of APs and foods. In addition, BMSB and BB were more attracted to improved Rocket trap equipped with solar fan than sole Rocket trap, and attraction was further increased to improved Rocket trap installed with solar fan

powered by battery and blue LED light than that of Rocket trap with solar fan only. Experiment related to trap distance showed that higher stink bugs were captured within 5 m far distance from the leguminous fields, and at border in case of apricot orchard. Accordingly, the types and hues of Rocket trap, food combinations, and trapping locations and distance are very crucial for successful monitoring and mass capturing of stink bug species.

Keywords: Stink bugs, Improved Rocket trap, Attractants, food combinations, Agro-ecosystems

CO029

SMART TECHNOLOGIES FOR THE DETECTION OF MIGRATING INSECTS: THE CHALLENGES OF AUTOMATIC COUNTING AND ID

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The Rothamsted Insect Survey (RIS) has been sampling migrating insects using its 12.2 m suction traps since 1964. In doing so, the RIS has amassed over 60 million records in that time, annotating species using classical taxonomy. In the last five years, there has been an exponential interest in real-time detection of insect threats using so-called ‘automatic methods’. Researchers have used electronics to explore the possibilities of detection and have focussed efforts on radar, lidar, camera traps with image analysis, acoustic and optical methods, all with varying degrees of success. In this presentation, we discuss the challenges that face a monitoring network with a large infrastructure and a standardised time series with fixed protocols. We show how a mobile ‘pop-up’ trap can be made to complement the existing network and how these and the 12.2 m traps can be enhanced with optical methods to detect the bioflow and estimate the total count of insects. This automation, in collaboration with the University of Hertfordshire, builds on previous research in which we were able to accurately estimate terminal velocities of spiders in freefall and photograph their posture during descent. We then focus attention on monitoring aphid pests at the species level and discuss the challenges of accurately identifying these insects flying just above crop height using in-field electronics. Given that there are more than 600 species in the UK and that these are difficult to differentiate between species even with a microscope, the challenges for automatic detection seem fraught with difficulty. However, a promising number of parameters are suggested but we argue that these should be applied in combination rather than relying on a single behaviour, such as wing beat frequency, which is most likely to fail if used in isolation.

Keywords: Migration, aphids, automatic identification, sensors, long-term experiment, suction trap, wing beat frequency

CO030

ASSESSMENT OF THE RISK POTENTIAL FOR THE OCCURRENCE OF *DROSOPHILA SUZUKII* CONSIDERING THE HABITAT AND THE HIBERNATION CONDITIONS

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Recent scientific research shows that the occurrence of *Drosophila suzukii* in a particular area correlates with the habitat structure. Woodlands and hedges with uncultivated close to cultivated host fruits providing particularly suitable habitats for *D. suzukii*. In addition to alternative host fruits, these areas have more favorable environmental conditions (higher humidity and moderated temperature regimes) for hibernation. Therefore the main question in our study was whether it is possible to statistically identify high risk areas/habitats for fruit infestation by *D. suzukii* out of general available weather data (hibernation conditions) and geodata (habitat structure) and to calculate a risk potential as input for decision support systems. To analyse how *D. suzukii* population is varring in relation to habitat structure and weather conditions in winter, monitoring data of adult flies were collected yearlong using vinigar traps. To find correlations between habitat structure, weather conditions in winter and captured adult flies in the traps the three datasets were geostatisically analysed (joined, merged and clipped). Afterwards the weight of the weather conditions and the weight of the amount of woodlands, hedges, *e.g.* on the quantity of caputred flies were statistically analysed by multiple binary logistic regression. The analyses showed a significant influence of the longest period of cold days (Tmax <8°C) in winter on the occurrence of *D. suzukii* in May, June and July. Furthermore, there were significantly positive influences of the amount of hedges in the area surounding the trap. In a first independent validation a correct classification of the predicted risk potential in the months May to July between 70 and 86 percent was reached. With the predicted risk potential it will be possible to improve the timing of monitoring and pest control of *D. suzukii* for the beginning of the season.

Keywords: *Drosophila suzukii*, influence habitat structure, influence weather conditions, risk potential, decision support systems

CO031

VIBRATIONAL MATING DISRUPTION: STATE OF THE FIELD, PERSPECTIVES AND COMPARATIVE ANALYSIS WITH CLASSIC PHEROMONE MATING DISRUPTION

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When talking about insect mating disruption, it is common to associate the concept exclusively to pheromones. However, in the last few years a new approach to disrupt the mating behavior of insect pests has been proposed and is quickly gaining recognition in the scientific community and beyond: the vibrational mating disruption (VMD). This approach relies on the use of substrate borne vibrations to interfere with vibrational signals emitted by insects during the process of pair formation. Biotremology is a new discipline that studies this kind of communication. In the course of approximately 10 years, the VMD progressed from simple theoretical concept to practical open field experimentation. In 2017, the first vibrational vineyard in the world was established in San Michele all’Adige for control of the leafhopper *Scaphoideus titanus*. The development will require years of field observations and of monitoring of both target and non-target species, also to assess possible ecological impacts of the method. Meanwhile, we made a careful analysis of the 50 years of experience with pheromone mating disruption to outline possible advantages and disadvantages of applying VMD to commercial crops, and for indicating weaknesses, strengths, similarities and differences. We examined both behavioral /physiological characteristics of the different target species and peculiarities of the two methods. In detail, we analyzed the following parameters: (1) sensorial modality and signal specificity; (2) searching behavior; (3) signal active space; (4) male rivalry behavior; (5) spatial dispersion; (6) insect phenology;

(7) mechanisms of action; (8) efficacy assessment. Our conclusion is that although a considerable amount of research still is needed (*e.g.* protocols, technology and standardization), VMD is becoming a feasible and applicable alternative to commercial solutions. In addition, we propose the use of the term “semiophysicals” as analogue of “semiochemicals” when referring to vibrational signals.

Keywords: Biotremology, vibrational communication, substrateborne signals, IPM

CO032

ANALYSES OF THE POPULATION STRUCTURE OF *RHOPALOSIPHUM PADI* LINNAEUS (HEMIPTERA: APHIDIDAE) IN THE UK AND THE VALUE OF THE ROTHAMSTED INSECT SURVEY ARCHIVE FOR GENOMIC ANALYSES

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The Rothamsted Insect Survey (RIS) has been monitoring aphid populations in the UK for over 50 years. Insect catches dating back to 1974 are held at the RIS archive and are available for scientific research. In the present study, we assess the usefulness of aphid samples of the RIS preserved in 95% ethanol – 5% glycerol as a source of DNA for temporal genetic and genomic analyses of aphid temporal dynamics. To investigate the effect of time of storage on DNA yield, we performed extractions from 156 individuals of the bird cherry — oat aphid, *Rhopalosiphum padi*, collected in 2010 (n=40), 2013 (n=44) and 2016 (n=72) and compared the amount of DNA obtained. We have sequenced these samples using a genotype-by-sequencing (GBS) approach and used multi-locus genotype analyses to explore the population structure of the R. padi in the UK. Results suggest low to moderate levels of genetic differentiation between locations in the north and south of the country, although they most likely constitute one single population. The population structure of the species has implication for its surveillance and control which will be discussed.

Keywords: Aphids, population genetics, genotype by sequencing, genomics, pest control

CO033

ADDING VALUE TO CULICOIDES SURVEILLANCE TRAPPING

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Biting midges, *Culicoides spp.* (Diptera: Ceratopogonidae), are important vectors of livestock viruses. Following the outbreak of bluetongue serotype 8 in Europe between 2006 and 2009, Culicoides surveillance programmes were initiated to identify vector-active periods, in accordance with European Commission regulation 2007/1266/EC. In Northern Ireland, trapping was conducted at 14 locations over a 4-year period (2008–2011 inclusive), resulting in over 750, 000 midges being collected. This represents a valuable resource for elucidating midge biology. The data were used to determine midge phenology, habitat preferences, effects of weather and influence of animal housing on midge prevalence. A similar approach could be used to extract more information on Culicoides ecology from surveillance datasets held in other countries. Brief mention will also be made of adding value by monitoring daily flight activity by use of time-sorting devices attached to standard traps.

Keywords: National surveillance, biting midges, habitat preference, time-sorting device

CO034

A SURVEILLANCE NETWORK FOR ASIAN HORNET, *VESPA VELUTINA* LEPELETIER (HYMENOPTERA: VESPIDAE), IN VENETO REGION (ITALY)

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After its first introduction in Italy in 2012, the Asian hornet (*Vespa velutina*) was reported in Veneto Region (northeastern Italy) in 2016. A surveillance program has been activated by the Regional Agriculture Department in collaboration with the National Reference Centre for beekeeping of the Istituto Zooprofilattico Sperimentale delle Venezie, with the aim to assess the presence and spreading of this invasive species. In order to select the apiaries to be involved in the program, geospatial data were analyzed and a statistical analysis of the distribution of apiaries was performed. In the area was defined a cells-divided grid and the number of apiaries for each cell was calculated; three classes of apiaries density per cell were defined (high, medium and low apiary density/cell). Cells with high apiary density were considered as a sampling unit, whereas medium and low apiary density cells were grouped together (two and four cells respectively) standing for a sampling unit. Overall, 229 cells were defined: 117 with high density and 112 with medium-low density grouped. In each of them, an apiary was selected as target where to place wasp traps (TapTrap®) to monitor the presence of the Asian hornet. The results were recorded in a database specifically created. Moreover, a web application was implemented with a “mobile first” approach, easy to use by smartphone and tablets, in order to quickly collect field and geospatial data and to be able to share geographical-related information. In total, 230 apiaries were monitored by traps and 1,728 apiary visits were recorded (on average 7.5 visits/apiary). None of the monitoring sites revealed *V. velutina*. However, in the light of Regulation (EU) No. 1143/2014, the monitoring for the presence of this invasive alien species on the territory requires a protracted application beyond a single beekeeping season in order to consolidate the results achieved.

Keywords: *Vespa velutina*, surveillance program

CO035

NATIVE WOOD-BORING BEETLES TRAPPED AT PORTS-OF-ENTRY CAN HELP TARGET LIKELY INVADERS

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Trapping programs carried out at ports-of-entry are aimed at intercepting non-native species accidentally introduced within imported woody materials or as hitchhikers in containers. Nonetheless, several native species are commonly trapped along with non-native ones. Whether these native species originate from forests surrounding the ports or from other native areas and then conveyed to the ports through national maritime trade is still largely uninvestigated. We analyzed 3 yr of trapping records of native bark- and wood-boring beetles (Cerambycidae and Scolytinae) collected at 12 Italian ports and their surrounding forests to help elucidate these patterns and the possible human role in the movement of native species within their native biogeographic region. Several species that occurred either inside or outside their native Italian range were found in the monitored sites. The abundance of the species found outside their native range was most strongly linked to the amount of national imports arriving at the port where trapping occurred, suggesting that they were likely introduced to the monitored ports from other Italian ports. Species richness and abundance of those species found within their native range were most strongly associated with the amount of forest area occurring in the surrounding landscape, suggesting that they could have arrived in the ports from the nearby forests. All these native species can be then moved in woody materials associated with exports. This study demonstrated the importance of identifying and analyzing the pool of native species that can be trapped at ports, both from an ecological point of view, documenting range expansion of native species, and from a practical point of view, allowing the creation of lists of wood-boring beetles that can most likely be moved by trade.

Keywords: Wood-boring beetles, non-native species, ports-of-entry, surveillance

CO036

DEVELOPMENT OF AUTOMATED DEVICES FOR THE MONITORING OF FRUIT FLIES PESTS

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The monitoring of insect pests, based on adult trapping systems, is a part of IPM programs in many crops and used to set up correctly control operations. Using data obtained from traps to make management decisions is not an easy task and involves significant costs in equipment, materials and labour. Thanks to the spread and the low prices of information technologies, innovative capture devices, that include electronic sensors and connecting components, are increasingly being developed, allowing to face some of the problems in a context of precision agriculture. In this paper, we illustrate the state of the art related to this issue, providing insights on our experiences in the development of systems for the monitoring of fruit flies (Diptera Tephritidae), consisting of traps equipped with sensors for the collection of data in the field, devices for the remote transmission, and server accessible online to store information in geo-referenced databases. The sensors can be different and connected to each other in wireless or radio networks; for the count of the captured fruit flies, optical or infrared sensors are mainly used. The monitoring systems can be equipped with software for image interpretation and identification of the caught target flies (automatic trap), or a remote operator can count the trapped flies, by observation of the images coming from the trap (semi-automatic trap). The software can integrate a decision support system (DSS) module, which provides information on the risk of infestation, the actions to be taken (when, where, how to perform a control) and a real-time computer assistant, to manage pathways and variable inputs of biocides during a precision treatment. The development and future prospects of these devices are discussed, in relation to the technical reliability of the system, ease of use and costs, compared to traditional monitoring methods.

Keywords: Monitoring, precision agriculture, IPM, traps, sensors, fruit flies

Emerging Challenges in IPM

CO037

NEARLY 100 YEARS OF TRICHOGRAMMA RESEARCH IN GERMANY – A REVIEW OF FAILURES, SUCCESSES AND FUTURE PERSPECTIVES

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Considering the area of release (ha/year), egg parasitoids of the genus *Trichogramma* (Hymenoptera, Trichogrammatidae) are the most widely applied biological control agent in Germany. Regular releases are performed on more than 20.000 ha/year against the European Corn Borer, *Ostrinia nubilalis* (Lepidoptera, Crambidae). Other more small-scale applications are conducted against pests in vegetables, ornamentals and storage. First attempts with mass production and release of these wasps were already performed in the 1920s by Albrecht Hase and colleagues from different research institutions against various pests in agriculture and forestry. About 40 years later, Karl Mayer and Wolfgang Quednau reported in the "Trichogramma-problem" (Mitt. Biolog. Bundesanstalt 100, 1960) on biological attributes which appeared to be useful for species differentiation. At the same time, trials to use the wasps against the codling moth were re-initiated at the Institute for Biological Control in Darmstadt, Germany. Twenty years later, Sherif Hassan, entomologist at the Institute for Biological Control, pushed research on and application of these egg parasitoids forward by building an active network between scientists in Germany and many other countries. Also nowadays, research on life history, basic biological attributes, genetics and possibilities for use in biocontrol is done in several research institutions and universities, but also by beneficial producers of these tiny wasps with a long history of "big" success stories.

Keywords: Egg parasitoid, science history

CO038

WHAT IS GOING ON WITH THE PREDATORY MITE *PHYTOSEIULUS PERSIMILIS* IN DRY CONDITIONS?

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Predatory mites of the family Phytoseiidae are tiny organisms (0.2-0.5 mm long). Despite their small size, they contribute greatly to the success of augmentative biological control, as natural enemies of phytophagous mites and small insects. The predatory mite *Phytoseiulus persimilis* is probably

the best known example of a phytoseiid mite used for augmentative biocontrol. It was the first mass-reared predatory mite species, and it is still successfully applied worldwide to control spider mites (*Tetranychus urticae*). As most phytoseiid mites, *P. persimilis* is susceptible to low relative humidity, and its performance as a natural enemy can be lowered by dry conditions. Spider mites, on the contrary, perform well in dry conditions. Consequently, biological control of spider mites on crops grown in arid environments remains a serious problem, which is difficult to solve with the commercially available predatory mites. With the objective of selectively breeding a drought-resistant strain of *P. persimilis*, we focused on the effect of low humidity on the egg and the adult female life stages. We first assessed the effect of constant and variable relative humidity on the egg survival of five populations of this species. In a second experiment, we studied the oviposition behaviour of *P. persimilis* adult females under high and low humidity conditions.

Keywords: Augmentative biological control, abiotic stress, relative humidity, egg hatching rate, oviposition

CO039

A DECADE OF POPULATIONS GENETICS AND GEOMETRIC MORPHOMETRICS RESEARCH ON THE WESTERN CORN ROOTWORM IN SOUTHERN EUROPE: WHAT HAVE WE LEARNED AND WHERE TO FROM HERE?

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The western corn rootworm (*Diabrotica virgifera virgifera* LeConte) (Coleoptera: Chrysomelidae), is the worst pest of maize (*Zea mays* L.) in the United States, and its spread through Europe has caused a flood of research into its biology, ecology, and management under European environmental conditions. Research from our genetic work conducted over the past decade have focused on comparing historical and contemporary levels of genetic diversity and gene flow across southern Europe and investigated introduction routes from the USA. We have shown little to no genetic structure across large geographic distances indicating that in southern Europe the species essentially is part of one large population that each year advances its invasion front and edges further into Western Europe. Indeed other authors have since confirmed these results. While these results found and methods used were important they were not the outcome expected nor did the tools used prove to be a management panacea and as such we continued to test other tools for monitoring the pest with the ultimate goal of seeking out a cheaper, quicker and easier monitoring tool that would have use as a reliable population biomarker. Through the use of geometric morphometrics it has been possible to find discernible patterns in wing shape that related to in-field resistance (ie. crop rotation resistance, Bt maize resistance). We have found an affordable and accessible technique that reliably demonstrates that hind-wing shape and size differences can be used to reveal differences among rootworm populations, and thus can be used as a biomarker for resistance detection as part of a larger integrated resistance management strategy for the species. The results of our research have important implications for ongoing rootworm monitoring and management, particularly where genetic capabilities and monetary investment in such techniques may not be feasible (*e.g.* in developing countries).

Keywords: Western corn rootworm, population genetics, invasive species, geometric morphometrics

CO040

THE IMPACT OF THE BROWN MARMORATED STINK BUG *HALYOMORPHA HALYS* (HEMIPTERA: PENTATOMIDAE) IN KIWIFRUIT ORCHARDS: DAMAGE CHARACTERIZATION, EFFECT OF ANTI-HAIL NETTING AND SURROUNDING LANDSCAPE

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The Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae), is a polyphagous pest native to eastern Asia, and an invasive species in Europe and North America. In invaded areas, this insect is becoming a primary pest in fruit orchards. Plants belonging to *Actinidia spp.* (kiwifruit) are reported as host plants in its native area, but little is known about the impact of the pest on kiwifruit in invaded areas. Here, we assessed the impact of the BMSB on kiwifruits *A. chinensis var. deliciosa* in cage trials and field observations. We also evaluated the effect of anti-hail netting and landscape traits on the presence of the BMSB within orchards. Infestations by the BMSB resulted in damaged fruits during maturation and induced the development of corky tissue in the pulp, especially in the upper part of the fruit. The damaged fruit can shrivel and drop before harvest. Higher infestation levels were found in orchards close to semi-natural habitats. Pest distribution within orchards were characterized by an edge effect. The presence of anti-hail netting was able to reduce infestation level and the lower numbers of BMSB was reflected also in lower proportion of damaged fruits, in particular in the center of the orchards. The implications of these experiments are discussed in regards of integrated pest management of this invasive pest.

Keywords: *Halyomorpha halys*; Brown Marmorated Stink Bug; kiwifruit; *A. chinensis var. deliciosa*; anti-hail netting; landscape effect

CO041

OLFACTORY RESPONSES OF *HYALESTHES OBSOLETUS* TO ELICITOR-INDUCED VOLATILES IN GRAPEVINE PLANTS

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Hyalesthes obsoletus Signoret (Hemiptera: Cixiidae) is considered to be the principal vector of ‘Candidatus *Phytoplasma solani*’, which is the causal agent of the grapevine yellows “Bois noir (BN)” in European vineyards. This planthopper is a polyphagous species living mainly on wild and cultivated herbaceous plants often hosting the phytoplasma. The life history of *H. obsoletus*, its affiliation to wild host plants, and its erratic feeding behavior on grapevine impede the effective control of this planthopper. Among alternatives to synthetic pesticides, a good interest was gained by the application of resistance inducers to control plant pathogens and insect herbivores. In a recent study, the activity of some elicitors in inducing recovery of BN infected grapevines was evaluated, and encouraging results were provided by benzothiadiazole (BTH) and by two different formulations containing glutathione plus oligosaccharides (GO1 and GO2). In this study, Y-tube olfactometer bioassays were designed to investigate *H. obsoletus* behavioral

responses to volatile emitted by grapevine shoots sprayed with these three commercial formulations containing BTH, GO1 and GO2. Each diluted formulation was applied at 3 different times (0, 2 and 7 days before bioassays). A volume equivalent to 1,000 l/ha of deionized water was applied to control plants to a run-off point, at the same times of treatments. The number of planthoppers tested was 32 for every treatment (total n=288 specimens). Our study showed that BTH-treated grapevine shoots were significantly repellent for *H. obsoletus* when applied one week before insect bioassays. Moreover, planthoppers were significantly attracted to volatiles emitted by grapevine shoots sprayed with GO2 formulation the same day of bioassays, when compared to the control. No behavioral responses have been elicited by treatments with GO1. The results of this study could be used as part of a integrated pest managment strategy to control *H. obsoletus*.

CO042

MECHANISMS MEDIATING FALSE CODLING MOTH MATING DISRUPTION, USING POINT-SOURCE PHEROMONE DISPENSERS

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Mating disruption is the broadcasting of female pheromones within an agricultural system, so as to interfere with or decrease the reproductive potential of the pest insect. Seen as a sustainable, non-chemical management method, it is used in citrus orchards against the false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick). Although research to support its efficacy against FCM is limited, even more surprising is the lack of understanding of basic physiological and behavioural questions, such as what mechanisms drive success or failure. Recent work has indicated that Lepidoptera are disrupted either competitively or non-competitively. No studies have yet been conducted on FCM to determine its disruptive mechanism. However, studies conducted on closely related species have proposed and critically evaluated a framework along with a dichotomous key for distinguishing between the two mechanisms. This provides a protocol for identifying the underlying disruption mechanism in other species. Here we examined the dosage-response profile as well as the optimal dose from multiple mark-release-recapture (MRR) experiments using sterile FCM. Stone fruit and table grape blocks were treated with increasing levels of pheromone dispenser densities which allowed the level of disruption experienced (FCM caught in treated/untreated blocks) to be calculated. The difference between the recapture rates from multiple MRR experiments was analysed and will help identify the disruption mechanism involved. The efficacy of mating disruption will be discussed and the mechanism that drives its success/failure identified. Such identification is vital to the success of the implemented control, which depends on the underlying behavioural mechanisms of the pest insect.

Keywords: Sustainable control, dosage-response profiles, IPM, Tortricidae

CO043

IMPACT, SURVEY AND CONTROL OF THE BEECH LEAF MINING WEEVIL, *ORCHESTES FAGI*, AN INVASIVE PEST OF AMERICAN BEECH IN NOVA SCOTIA, CANADA

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The beech leaf mining weevil, *Orchestes fagi* (Coleoptera: Curculionidae), native to Europe, was discovered infesting American beech in Halifax, Nova Scotia, Canada in 2012, but was likely established there 5–7 years prior to its discovery based on anecdotal reports of typical signs of damage to foliage. After several consecutive years of heavy infestation by the weevil (*i.e.*, 70% of leaves with mines), many mature American beech trees have died, with >85% mortality in natural stands and about 40% mortality of trees in residential Halifax properties. The risk of human-assisted spread of the weevil via movement of logs and firewood is high because adults overwinter under bark scales on the trunks of trees. We present data from experiments designed to develop tools for survey and control of *O. fagi*. The most effective trap and lure to date is a bright yellow sticky trap baited with geranyl-p-cymene (a host volatile emitted from beech buds at the time of budburst, when mating and oviposition occurs), and placed in the upper canopy of beech trees. Stem injection of mature beech with TreeAzinTM, produced from azadirachtin extracts from seeds of the neem tree, in the fall or early spring significantly reduced larval survival and damage to foliage. Extremely low–nil levels of parasitism of *O. fagi* have been observed in Nova Scotia, suggesting classical biological control may be a viable long-term strategy if species-specific parasitoids can be sourced in Europe.

Keywords: *Orchestes fagi*, Beech leaf mining weevil, impact, survey, control

CO044

THE USE OF EXCLUSION NETS FOR PEST MANAGEMENT IN FRUIT ORCHARDS IN NW ITALY

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In the last years, many chemical products were banned in the European Union, posing new challenges for the control of crop pests. In this context, insect exclusion nets represent a promising eco-friendly alternative strategy for plant protection. In the frame of the LIFE+ SU.SA.FRUIT project (LIFE13 ENV/HR/000580), experimental trials were carried out in NW Italy in 2016-2017, in order to assess the efficacy of a pearl photo-selective net (mesh 2.4x4.8mm) in containing key and emerging insect pests in peach and apple orchards. In two peach and two apple orchards, plots of 16-20 trees were caged with the net from petal fall until harvest time, while similar plots without nets were used as controls (three netted and three un-netted plots in each orchard). Since the closure of the nets, no insecticide treatments were applied in the netted and un-netted plots. The presence and abundance of key pests, such as *Cydia pomonella* and *Grapholita molesta*, and of new exotic pests, such as *Halyomorpha halys*, were assessed by means of pheromone traps all along the season inside and outside the nets. The damage on fruits at harvest time was recorded in the netted and un-netted control plots as well as in further three plots per orchard without net but insecticide-treated by the growers. The net coverage allowed to obtain a significant reduction of pest populations and as a consequence of damaged fruits (up to 45% reduction in peach orchards) in comparison with the un-netted control plots and with the insecticide-treated plots as well. At the same time, no negative effects on fruit quality were recorded. The exclusion nets are therefore a very promising and sustainable tool for the management of important fruit pests.

Keywords: *Cydia pomonella*, *Grapholita molesta*, *Halyomorpha halys*, peach, apple, fruit damage

CO045

IDENTIFYING POTENTIAL PEST THREATS TO SITKA SPRUCE PLANTATIONS IN IRELAND

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Sitka spruce (*Picea sitchensis*) is the most important commercial forestry species in Ireland. Over fifty percent of the Irish forest estate is made up of Sitka spruce plantations and forestry in Ireland contributes €2.3 billion to the economy each year. Forest pests can cause huge economic losses and impact on ecosystem services, and therefore it was considered high priority to identify potential pest threats to Sitka spruce in Ireland. A pest list was created containing over a 1000 pests and potential pests of spruce globally. Clustering analyses have been previously used to predict the top invasive species threats to the EU. In this research, two methods of clustering analysis Hierarchical Clustering (HC) and Self Organizing Maps (SOM), were applied to the pest list in order to analyse the geographic distribution of the pests by invertebrate order. As well as providing a list of top species most likely to be introduced to Ireland, the HC and SOM also clusters Ireland with those countries from which invasive pests are most likely to originate. Plants for planting are a very high risk pathway for the introduction of pests to new regions. A review of invasive forest pests in the USA concluded that nearly 70% of damaging forest insects and diseases introduced to the USA most likely entered on imported live plants. The pest list identified 874 pests and potential pests of Sitka spruce that could enter Ireland on the plants for planting pathway. The pest list was then further prioritised, and the risk of other 220 pests analysed in a pathway pest risk analysis. The pathway analysis identified that certain coniferous species, which are currently not regulated by the EU, could potentially provide pathways of entry for pests that pose a risk to coniferous forestry across the EU.

Keywords: Sitka spruce, clustering analysis, pest risk analysis

CO046

THE SOUTH AMERICAN TOMATO PINWORM IN THE MEDITERRANEAN: CURRENT PEST STATUS AND MANAGEMENT STRATEGIES

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The Neotropical moth *Tuta absoluta* (Lepidoptera: Gelechiidae) remained confined to the its native region up to 2005. After being reported in Spain in 2006, it spread rapidly throughout Afro-Eurasia and became a major threat to world tomato production. The remaining major tomato-producing regions of the world (*i.e.*, China, Mexico, and the US) are at high risk of being invaded. The widespread insecticide use for its control has caused selection for insecticide resistances, as well as undesirable effects on key beneficial arthropods. For its high demographical potential and economic relevance, *T. absoluta* can be effectively contained only by integrating several control strategies. Augmentation and conservation biological control, relying mainly on omnivorous mirid predators, has proved successful. Larval parasitoids showed potential for further development of sustainable control, but their biocontrol services and applications are marginal. Novel biopesticides, *e.g.* botanical extracts, insect pathogens, mineral formulations, are increasingly being used. Controversial results were obtained by mating disruption application; the moth sexual pheromone is instead greatly and effectively used alone or together with light and or color, as attractant in mass trapping applications that contribute to the sustainable control of this pest. Several resistant mechanisms in tomato plants have been characterized, but no resistant or tolerant tomato varieties are commercially available yet. The implementation of sustainable pest control programs, which is also supported by the current European regulation on crop protection, is essential to manage this key pest and to prevent further spread worldwide.

Keywords: Biocontrol, biopesticides, invasive species, IPM, insecticide resistance

Session 2.
Behaviour

Mechanisms of Learning and Memory

CO047

IMPRINTING ALTERS DROSOPHILA SEX PHEROMONE RESPONSE

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Pheromones are chemical signals that induce innate stereotypical responses in individuals of the same species. In the vinegar fly *Drosophila melanogaster*, the most intensively studied pheromone is 11-cis-vaccenyl acetate (cVA), which is synthezised in the male ejaculatory bulb and is transferred to the female during copulation. cVA inhibits male courtship of mated females, and when transferred into the food substrate during egg-laying, attracts male and female flies, stimulates female mating and plays a role in stimulating male-male territorial aggression. We detected the presence of cVA on eggs and measured its negative influence on the courtship behavior of males that hatched from these eggs. After determining the dose-dependent effect of cVA on male courtship suppression, we then studied the effect of cVA when present on the egg or larva. Males that hatched from eggs that were either not exposed to maternal cVA during embryonic and 1st instar larval stages, or were laid several days after mating, showed the strongest effects. Adding synthetic cVA to washed eggs did not rescue the effect of maternal cVA. This indicates that the cVA-induced inhibition of male courtship behavior largely depends on imprinting by cVA combined with other maternally-transmitted factor(s).

Keywords: Sex pheromone, Imprinting, *Drosophila melanogaster*, coursthip behavior, 11-cis-vaccenyl acetate, cVA

CO048

GENOMIC AND REGULATORY BASIS OF IMPROVED LEARNING ABILITY IN PARASITOID WASP SELECTION LINES

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Despite great progress in revealing genetic mechanisms underlying behaviour, the genetic architecture shaping natural variation in learning and memory dynamics remains poorly understood. Here we identify candidate regulators of learning behaviour in the parasitic wasp Nasonia vitripennis. We selected for improved associative learning and memory formation in associating a colour with host presence, using replicated pairs of lines. Using whole-genome resequencing, we identified consistent signatures of improved learning across the genome. Allele frequency diverged consistently between the selected and control lines in 118 single nucleotide polymorphisms (SNPs), clustering in 51 distinct genomic regions containing 128 genes, several of which have functions in neural plasticity and cognitive processes. Remarkably, the majority of SNPs were found in regulatory regions, suggesting an important role for gene expression evolution. We therefore sequenced the transcriptomes of control and selected lines, and identified 35 differentially expressed transcripts of large effect, with the strongest response for two alternative transcripts of the same locus. Only one gene showed both sequence and expression divergence as a result of selection. Taken together, there is a reasonably modest set of conservatively identified candidate genes, where sequence and regulatory evolution affect learning behaviour in Nasonia.

Keywords: Selection experiment, associative learning, memory formation, gene expression, whole genome resequencing

CO049

SYNAPTIC DE-CORRELATION IN THE MUSHROOM BODY UNDERLIES ODOR LEARNING IN DROSOPHILA

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Synaptic de-correlation in the mushroom body underlies odor learning in Drosophila. Plastic changes in synaptic transmission between neuronal ensembles represent the neuronal substrate underlying learning and memory formation. This process is complex: In the course of classical, associative conditioning a huge diversity of sensory stimuli and their combinations can be learned as indicative for relevant positive or negative outcomes. Since sensory stimuli are typically encoded as sparsely distributed activity across assemblies of many neurons, it is challenging to determine which and how individual synaptic connections change to acquire a stimulus-specific memory. Here we used *in vivo* single cell calcium imaging in Drosophila to monitor how associative olfactory learning modifies odor-evoked activity in synaptic boutons along individual axons and across many neurons. We focused on axonal boutons of γ-lobe Kenyon cells of the mushroom body, a brain structure to which associative olfactory learning could be localized. We confirm that axonal compartments of individual Kenyon cells rather than entire neurons act as functional, stimulus-encoding and memory-forming units. Furthermore, we found that albeit associative learning induced bi-directional changes in synaptic bouton activity, their net odor-evoked calcium activity remained constant. However, odor-evoked synaptic bouton activity within and across single Kenyon cells significantly de-correlated as a result of associative learning, and specifically for the trained odor. This reveals a novel principle how associative memories are formed.

Keywords: Learning and Memory, *Drosophila melanogaster*, Synaptic Plasticity

CO050

INTEGRATION OF PARALLEL OPPOSING MEMORIES UNDERLIES MEMORY EXTINCTION IN DROSOPHILA

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Learned fear responses can be suppressed by experiencing predictive cues without the expected punishment, a process called memory extinction. Here we show that extinction of aversive memories in Drosophila requires rewarding dopaminergic neurons, suggesting that omission of punishment is remembered as a positive experience. Functional imaging revealed the co-existence of two opposing memory traces after extinction, the original aversive memory and a new extinction reward memory, in different places in the mushroom body output neuron network. Therefore, we argue that flies determine the likelihood of learned expectations by accumulating and integrating memories of conflicting events.

Keywords: Learning, memory, re-evaluation, extinction memory, Drosophila, dopamine

CO051

HANDLING OF MORPHOLOGICALLY COMPLEX FLOWERS BY BUMBLEBEES

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Learning complex motor tasks involves expending considerable time and energy. Nevertheless, foraging animals often persist and learn to acquire rewards from food sources that are difficult to access, despite their low initial profitability. Bees that handle morphologically complex flowers present an interesting system to explore this little understood phenomenon. In this study, we examined how complex flowers are handled by bumblebees (*Bombus terrestris*) when presented along with simple flowers in a flight room. We specifically examined whether a) bumblebees have any innate preferences for complex flowers, b) they specialize on simple flowers, given equal food rewards c) high rates of improvement in accessing rewards in complex flowers lead to increased preference for these flowers d) individual bees exhibit constancy (runs of consecutive visits to each flower type). We manipulated the tubular flowers of Tecomax'Orange Jubilee' (Bignoniaceae) to make them simpler, while intact flowers were considered complex. In a choice assay, bees were given a 4x4 array of equally rewarding simple and complex flowers. 62% of naïve foragers chose a complex flower on their first visit. Bees took significantly longer time to handle complex flowers, but also improved faster on them with experience. Despite quicker handling of simple flowers, experienced bees did not specialize on them but, on average, indistinctly visited both the flower types. Individual bees exhibited differential preferences towards complex flowers and varied in their constancy. Foragers that specialized on complex flowers predominantly collected nectar and not pollen when tested for short-term foraging preferences, regardless of body size and colony age during testing. Constancy to flower type decreased with colony age, but was not affected by flower-type preference or body size. Lastly, our ongoing experiments with *Lupinus pilosus* (Fabaceae), a difficult flower, will complement these findings and provide further insights on learning of complex flowers.

Keywords: Complex, Foraging, Flower handling

CO052

TRACE CONDITIONING - HOW INSECTS ASSOCIATE TEMPORALLY SEPARATED STIMULI

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Insects readily associate odors which co-occur with food. But they also associate odors which precede the food by several seconds. This type of learning is called trace conditioning. Trace conditioning requires a sensory odor memory (also called odor trace) that bridges the time between the odor stimulus and food reward. Notably, this time-bridging capability increases with experience, which could be explained by attention or higher order learning. The neural mechanisms of trace conditioning are unknown. Using Ca2+ imaging in Drosophila, we found persistent odor-evoked Ca2+ responses in the somata of Kenyon cells in the mushroom body (the memory center in the insect brain) that could encode a sensory odor memory. During trace conditioning, Kenyon cells decreased their responses to the associated odorant in specific compartments of the mushroom body, while in the same compartments, dopaminergic neurons that encode reward or punishment increase their responses to the associated odorant. These data suggest that Ca2+ in KC somata could serve as a basis for associations between temporally separated stimuli, and that response plasticity dopaminergic neurons encodes learning-induced changes in the odorant's predictive power.

Keywords: Trace conditioning, sensory memory, associative learning, honey bees, Drosophila

CO053

Abstract moved.

CO054

CELLULAR RESOLUTION MNEMONIC MECHANISMS IN DROSOPHILA

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Animals constantly reassess the reliability of learned information to optimize their behaviour. A mismatch between learned and perceived information, a prediction error, permits an adjustment of existing memories to adapt to unexpected changes in the environment. We found that omission of expected reward in Drosophila drives extinction learning via negatively reinforcing dopaminergic neurons. In contrast, a partial training trial renders reward memory temporarily labile and initiates memory reconsolidation, which requires different negatively reinforcing dopaminergic neurons and delayed action of positively reinforcing dopaminergic neurons. These data establish that recurrent and hierarchical connectivity between mushroom body output neurons and dopaminergic neurons enables memory re-evaluation driven by reward-prediction error. The functional diversity of dopaminergic neurons suggests that individual cells likely have unique input specificity and perhaps other physiological properties. I have used single-cell transcriptomics to assess potential heterogeneity of dopaminergic neurons.

Keywords: Memory, extinction, reconsolidation, dopamine, single-cell transcriptomics, Drosophila

Communication: From Signal Production to Perception

CO055

GOOD VIBES: A WASP SOCIAL PARASITE INTERCEPTS THE VIBRATIONAL COMMUNICATION SYSTEM OF ITS HOST

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In the most extreme cases of social parasitism, such as in the paper wasp host-parasite system, *Polistes dominula – Polistes sulcifer*, the obligate social parasite has lost the worker caste and it thus relies on its host social system and worker force to rear its own reproductives. In such cases the social parasite fitness depends on its ability to successfully integrate into the host colony and replace the dominant breeder, by intercepting the host communication system. As the host communicates through chemical, visual and vibrational sensory channels, the social parasite would benefit from exploiting each of them to achieve a complete host colony control. Indeed, in paper wasps, substrate borne vibrations are produced by body oscillations and have been suggested to play a crucial role in dominance hierarchies signaling and caste-fate determination. Here we investigated whether the social parasite, *Polistes sulcifer*, which is able to chemically integrate into the society of its host and to visually cheat the host, is also able to integrate into the host vibrational communication system. Thanks to a laser vibrometer we recorded the vibrations produced by abdomen oscillations of social parasite and host females, on both parasitized and control colonies. Our results show that both species produce substrate borne vibrations consisting in groups of repeated broad-band pulses. While frequency distribution largely overlaps, the vibrations produced by the social parasite are characterized by longer and more intense events compared to the host ones. Moreover, we found that host females on usurped nests performed shorter and less intense vibrations than those produced on queen-right colonies. Our results support the hypothesis that the social parasite intercepts the vibrational communication system of its host. This, in synergy with ritualized dominant behavior and chemically signaling, likely allows the parasite to successfully replace the host dominant breeder.

Keywords: Paper wasp, Polistes, abdominal wagging, social parasite, laser vibrometer, biotremology

CO056

TRANSCRIPTOMIC AND MORPHOLOGICAL EXPLORATION OF THE SENSORY SYSTEM OF *DROSOPHILA SUZUKII* OVIPOSITOR

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Drosophila suzukii is an invasive pest that lays eggs on undamaged ripening fruits, rather than in fermenting substrates like most other Drosophila species. It has evolved an enlarged and serrated ovipositor that enables gravid females to pierce intact fruit skin. The location and selection of the best oviposition site involves visual, olfactory, and tactile responses. Here we explored the sensory system of the ovipositor of *D. suzukii*, and we compared it to that of closely related species. We first sequenced the ovipositor transcriptome in *D. suzukii* and in three Drosophila species characterised by progressive changes in oviposition behaviour: annotation and comparison of genes encoding chemoreceptors revealed a set of gustatory and ionotropic receptors conserved among the four Drosophila species. We therefore tested the occurrence of chemosensory neurons in *D. suzukii* ovipositor using an anti-horseradish peroxidase antibody to target presence and shape of neuronal structures: results indicate that neurons associated with *D. suzukii* ovipositor spines are mechanosensory neurons. Further analysis of morphology and ultrastructure support the hypothesis that these structures are involved in mechanoreception. In conclusion, our study supports the presence of mechanoreceptors on the tip of the ovipositor of *D. suzukii* which are probably used to test the stiffness of substrates. However, we cannot exclude a role of ovipositor in chemoreception, since transcription of some chemoreceptor genes seems to be a feature common to Drosophila species.

Keywords: *Drosophila suzukii*, oviposition, chemoreception, mechanoreception, transcriptome

CO057

ODOUR PERCEPTION IN ANTS: THE ROLE OF INDIVIDUAL EXPERIENCE

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Ants heavily rely on chemical cues and signals in their natural environment. Odour processing is a complex phenomenon, especially when animals are confronted to olfactory mixtures, made of numerous components. A given component, such as a floral odour, may produce a certain response when encountered alone, but a different response when encountered in a mixture if, for instance, it is overshadowed by another component. Moreover, perceptual similarity between two odour stimuli may be shaped by experience. Carpenter ants dramatically improve olfactory discrimination after differential conditioning compared with absolute conditioning, showing that olfactory similarity is not fixed but depends on the individual's perceptual experience. Pheromones, besides their established role as releasers of stereotyped behaviours, may also act as behavioural modulators. In the context of nestmate recognition, ants previously exposed to formic acid (alarm pheromone) are more aggressive towards non-nestmates and less aggressive towards nestmates than control ants. Formic acid thus improves discrimination accuracy. This shows that nestmate discrimination ability is not a fixed, unchangeable property of an individual ant but it is rather a plastic phenomenon. Taken together, these examples suggest that individual plasticity and experience-dependent behaviour should not be underestimated in the study of chemical communication in insects.

Keywords: Chemical communication, Social insects, cuticular hydrocarbons, learning

CO058

INHIVE MONITORING OF ELECTROSTATIC FIELDS IN HONEYBEE COLONIES

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In honeybee colonies communication plays a major role for the foraging success and according to that for health of the whole colony. Besides communication via pheromones, movements of the whole body and especially the wings are used to transport information. Because the honeybees get electrostatically charged during flight it is possible to detect the change of the position of their electric field and thereby the kind of movement they perform. Electrodes were used to capture the electrostatic signals in different colonies on two combs each. The main advantage of this technique is that the colony could be kept in natural conditions because no light and space is needed for the recordings. Long term recordings are possible because bee wax doesn't reduce the quality what is a big advantage over sound recordings. In this study we have extracted the occurrence of the well studied waggle dances and stop signals to get information of the state of the honeybee colony. Further we quantified the non-communicaton behavior of fanning, which is used to optimize the inhive climate and to circulate pheromones. Additional sensors for temperature and humidity in and outside the hive were used to relate the occurrence of the detected behavior to the weather conditions. In study sides all across Germany data was collected to link the behavior and the state of the honeybee colonies to different environmental conditions.

Keywords: Honeybee, waggle dance, electrostatic

CO059

FAST ODOR INFORMATION CODING VIA NEURONAL RESPONSE LATENCIES IN THE HONEYBEE BRAIN

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Odorants are coded in the primary olfactory processing centers by spatio-temporal patterns of glomerular activity. The spatial patterns of odorant-induced responses has been extensively studied and were found to form a general odor code, conserved across individuals. However, the importance of temporal features for odor coding is still debated. Here, we studied the early dynamics of the odor code via fast two-photon calcium imaging of the honeybee antennal lobes. We find that the first information on the odorant quality is encoded in the firing onset latencies of antennal lobe output neurons. We show that latency ranks form a universal code, conserved across subjects. The latency code allows to blindly predict an odorant stimulus with the same accuracy as the response amplitude code. However, glomerular latencies and amplitudes appear to encode complementary stimulus information. Finally, the latency code is in very good agreement with behavioral data. Our results show that neuronal response latencies provide the first information for odor identification, anticipating the slower response amplitude code.

Keywords: Honeybee, Antennal lobe, Two-photon microscopy, Calcium imaging, Odor coding, Latency code

CO060

SEX PHEROMONE AND INFORMATION TRANSFER

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Sex phenomes may have evolved through natural selection and sexual selection. Under natural selection a stabilized selection is expected, narrowing the risk of mistaken mating with the wrong species. Sexual selection, however, may give rise to a variation in the pheromone characteristics among females, if it transfers information about the female quality and affecting male mate choice. We tested the hypothesis that the female produced sex pheromone transfers information about the female quality and males use this information to choose their nates. We manipulated fitness parameters of *Pectinophora gossypiella* female moths such as size, age and satiation levels. We analyzed the pheromone amount and its ratio in the female glands and tested the males' mate preference in an olfactometer and a wind tunnel. We found that the pheromone characteristics varied among females in various conditions and males distinguish among females by the information transferred via the pheromone, and preferred females in optimal condition over females that are under stress. Interestingly, in the wind tunnel experiments males preferred females in optimal condition regardless of their proximity to the searching males. The latter results may shed light on the transfer of pheromone in air.

Keywords: Sex pheromone, sexual selection, mate choice, condition dependent

CO061

THE MOLECULAR BASIS FOR WAGGLE DANCE COMMUNICATION IN THE HONEY BEE *APIS MELLIFERA*

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The waggle dance is a stereotyped behaviour that honey bees use to share information about the location of a new food source or a new home with other members of the colony. There are two key components in this behaviour: 1) the translation of the spatial information into a simple movement performed by the dancer; 2) the decoding of the dance operated by dance followers. Despite the wide range of experimental work performed on the honey bee waggle dance, we still know very little about the molecular mechanisms regulating this fascinating behaviour. In this study, we have adopted a transcriptomic approach to underpin the genetic regulation of both components of the waggle dance. We have performed the RNAseq analysis of mushroom bodies from bee dancers that were trained to artificial feeders placed in different locations, and from dance followers that followed these dances. We present the output of the RNAseq experiments in terms of differentially expressed genes, and also in terms of biological processes, metabolic functions and co-expression networks associated with these genes. Finally, we discuss our findings in the broader context of the molecular regulation of complex behaviours and social learning in animals.

Keywords: Honeybees, *Apis mellifera*, waggle dance, RNAseq, gene expression, brain, mushroom bodies, social learning, social behaviour

CO062

NOT SOLELY MORPHOLOGICAL MIMICS: IMITATION OF HYMENOPTERAN FLIGHT TRAJECTORIES IN *CLEARWING MOTHS* OF SOUTHEAST ASIAN RAINFORESTS

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Clearwing moths (Lepidoptera: Sesiidae), described mostly from pinned specimens, are known for their physical resemblance to hymenopterans. We found three species of bee mimics in Southeast Asian rainforests, flying in a zigzag trajectory, nearly indistinguishable from bees, whereas wasp mimics displayed a faster, straighter flight, like wasps. Three new species of *Clearwing moths* (*Heterosphecia pahangensis* Skowron, *Aschistophleps argentifasciata* Skowron Volponi and *Pyrophleps ellawi* Skowron Volponi) were described, including two bee mimics and one wasp mimic. The flight of bee mimics, bees, wasp mimics and wasps was filmed in their native habitat, in slow motion and digitized. All known parameters used in locomotor mimicry studies were calculated and compared. Our results are the first experimental evidence for the existence of behavioural mimicry in *Clearwing moths*.

Keywords: Batesian mimicry, Sesiidae, locomotor mimicry, new species

CO063

VIBRATIONAL COMMUNICATION NETWORKS: LEAFHOPPERS AS A CASE STUDY

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Communication via substrate-borne vibrations is not only the most widespread form of insect acoustic communication, but in evolutionary terms is also older than communication via air-borne sound. Leafhoppers (Hemiptera: Auchenorrhyncha: Cicadellidae) are small (0.2-1 cm) phytophagus insects and are with more than 22 000 described species one of the most speciose groups. Field studies revealed that they communicate in complex vibroscape and share vibrational channel with other conspecific and heterospecific signallers, as well as rivals and exploiters. Since in this group, mate recognition and location are mediated exclusively via vibrational signals, leafhoppers provide an ideal model system to study interactions shaping the evolution of this communication channel. More detailed work on such diverse, but hitherto largely neglected groups, is likely to provide invaluable empirical data to address some questions that are central to our understanding of communication in general. Sexual communication is based on a coordinated exchange of species- and sex-specific vibrational signals and male is searching for a replying stationary female. Species from the genus *Aphrodes* served as a model group to study interactions in communication network where signals used in sexual communication between partners also affect other potential receivers living in the environment. We studied ecological context in which vibrational signalling occurs in the field in order to obtain relevant information on natural vibrational communities and investigated in more detail the mechanisms underlying communication strategies observed in the field. We also examined the influences of social environment on leafhopper communication strategies.

Keywords: Vibrational communication, vibroscape, biotic noise, leafhoppers

Reproductive Strategies of Entomophagous Insects

CO064

DIFFERENTIAL RESPONSES OF *TRISSOLCUS JAPONICUS* TO PLANT VOLATILES INDUCED BY THE INVASIVE HERBIVORE *HALYOMORPHA HALYS VS* THE BENEFICIAL PREDATOR *PODISUS MACULIVENTRIS*

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The brown marmorated stink bug, *Halyomorpha halys*, a polyphagous pest native from eastern Asia, has recently invaded North America and Europe, resulting in serious damages to several agro-ecosystems. The egg parasitoid *Trissolcus japonicus* is the most effective egg parasitoid of *H. halys* in its native area and might be a candidate for classical biological control. However, laboratory host range of *T. japonicus* includes *Podisus maculiventris*,

a Nearctic predatory stink bug used as a biological control agent. We tested *T. japonicus* responses in Y-tube olfactometer to stink bug-induced plant volatiles and to volatiles emitted by eggs and by reproductively mature males and females of *H. halys* and P. maculiventris. Volatiles from adult and egg mass of both *H. halys* and P. maculiventris were not attractive to *T. japonicus*. In contrast, tomato plants subjected to oviposition and feeding by *H. halys* were significantly attractive for the wasp, whereas plants subjected only to feeding were not attractive. Plants subjected to oviposition and/or feeding by *P. maculiventris* were not attractive. Therefore, *T. japonicus* was able to exploit *H. halys* oviposition-induced plant volatiles, even in a plant-herbivore system (*i.e.* tomato-*H. halys*) with which it did not co-evolve. In contrast, in no case the wasp was attracted by P. maculiventris, providing hope for future application in biological control.

Keywords: Invasive species, *Halyomorpha halys*, *Trissolcus japonicus*, egg parassitoid, plant volatiles, predatory stink bug, *Podisus maculiventris*

CO065

IS PARASITOID SUCCESSFUL REPRODUCTION A SPORADIC EVENT IN NATURE?

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Parasitoids are model organisms in behavioural and evolutionary ecology, mostly because their reproductive behaviour is directly connected to fitness returns, facilitating the measurement of the costs and benefits of foraging and oviposition behaviours. Implicitly, an egg laid is most often assumed to result in an offspring produced. However, immature parasitoids face an array of threats during their development. Parasitoid successful reproduction can be impacted by (i) specific mortality factors (*e.g.* super- and multiparasitism, hyperparasitism), most of the mortality factors associated to the host (*e.g.* predation, pathogenic infection, starvation), and (iii) non-reproductive effects of parasitoids on their hosts (*e.g.* mutilation, aborted parasitism, host feeding). Parasitoid reproductive strategies are therefore likely to be shaped by a high probability of substantial mortality occurring between oviposition and adult emergence, as proposed by Peter Price in the early 70's. This view of parasitoid ecology is relevant for the study of host-parasitoid population dynamics and biological control.

Keywords: Parasitoid, reproduction

CO066

DOES KIN RECOGNITION AFFECT AGGRESSIVENESS AND OUTCOME OF CONTESTS BETWEEN FEMALES OF THE SOLITARY PARASITOID WASP, *EUPELMUS VUILLETI*?

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Kin recognition, defined as the ability to differentiate genetically related from unrelated individuals, is known to play a key role in the expression of altruistic behaviours. This recognition can operate by different mechanisms based on genetical and environmental (familiarity) cues that individuals perceive from their conspecifics. In parasitoid hymenoptera species, hosts represent a limited resource for which females may fight for food and to lay their eggs. Although the ability to treat kin differentially may influence interference between parasitoids competing for host, only few studies have tested this empirically. Here, we explored the effect of relatedness and familiarity on the intensity (aggressiveness) and resolution of conflict in females of the solitary ectoparasitoid wasp, *Eupelmus vuilleti* (Hymenoptera: Eupelmidae). Females parasitize and feed upon larvae and pupae of *Callosobruchus maculatus* (Coleoptera: Bruchidae), which infest seeds of *Vigna unguiculata* (Fabaceae). When several females are simultaneously present on a patch, they tend to protect the host that they exploit by displaying agonistic behaviours towards conspecific competitors. In sub-Saharan Africa, cowpea seeds are traditionally stored in granaries where populations of both hosts and parasitoids can reach high densities, leading *E. vuilleti* females to experience intense intraspecific competition with both kin and non-kin conspecifics. Thanks to a 2x2 factorial design, we made sibling or non-sibling juveniles develop on hosts within the same seed or different seeds. We then investigated if i) the relatedness among adult females (full sibling *vs* non-sibling), ii) the seed containing the host (same *vs* different seeds) and iii) the interaction between both factors affect female aggressiveness and outcomes of contests.

Keywords: Agonistic behaviours, conflict resolution, relatedness, familiarity

CO067

CAN ENFORCED HOST SHARING BY SUB-SOCIAL PARASITOIDS ELICIT TRAITS OF HIGHER SOCIALITY?

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Some parasitoid Hymenoptera are sub-social: each *Goniozus nephantidis* (Hym: Bethylidae) mother provides maternal care by guarding her host and developing brood against other foraging females. Females engage in decisive contests, with losers excluded from the vicinity of the host, and offspring groups are thus produced by only one mother. Microcosm experiments show that, even if every female is in possession of a host, females that are not isolated from each other during brood production mutually interfere and per capita offspring production is reduced. Here we report how mutual interference declines with density in microcosms and explore its behavioural mechanisms. Further, we report the effects of varying the number of 'foundress' females that are presented with a single host and then confined within its vicinity. Host size, foundress number and foundress relatedness were all varied. We find that, under these circumstances, multiple females will reproduce together on a single host (a trait of higher sociality). Although larger groups of foundresses typically produce larger groups of offspring, the *per capita* production is greatest when females are alone. Single females are fully able to supress even the largest host we were able to present them with. Hymenopteran sociality is often explained by kin selection arguments. We find several effects of inter-foundress relatedness, including that groups of siblings produce offspring with much more female-biased sex ratios than groups of non-siblings. We draw comparison with natural quasi-sociality in another bethylid genus, *Sclerodermus*, in which *per capita* reproduction on large hosts, that are difficult to suppress, is enhanced by multi-foundress groups cooperatively tending large broods of offspring.

Keywords: Mutual interference, Sociality, Sex ratio

CO068

INTRASPECIFIC AND INTERSPECIFIC COMPETITION BETWEEN *TRISSOLCUS GRANDIS* AND *T. VASSILIEVI* (HYM., PLATYGASTRIDAE) FEMALES IN MULTI-PATCH ENVIRONMENT

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Trissolcus grandis (Thomson) (Tg) and *T. vassilievi* (Mayr) (Tv) (Hym. Platygastridae) are prevalent egg parasitoids of common sunn pest (CSP), *Eurygaster integriceps* Puton (Hem. Scutellerida), the most serious insect pest of cereals. Which one is more successful competitor is the object of this study. Competition between female parasitoids with competitors of either the same or the other kind was investigated at different parasitoid densities in a multi-patch environment. Eight combinations of female wasps were confined for 6h in an arena including five patches, each consisted of 1, 2, 4. 8, or 15 host egg clutches randomly distributed around the arena. Treatments were as “1 Tg alone”, “1 Tv alone”, “8 Tg”, “8 Tv”, “4Tg+4Tv”, “16Tg”, “16 Tv”, and “8Tg+8Tv”. Three replications were included per treatment. The host eggs were reared to emerge parasitoid or host progeny. The parasitism rate was estimated by counting emerged wasps of any kind. The searching rate of each species was estimated by Nicholson (1933) model for an arena. The results revealed that *T. grandis* has been the most serious competitor. Searching rate declined by wasp density more rapidly in *T. grandis* than *T. vassilievi*, but *T. grandis* displayed a significant increase in searching rate when competed with competitors of the other kind. This was inverse in T. vassilivi. Parasitism of *T. vassilievi* sharply declined in presence of *T. grandis*. These results may partly explain that why *T. grandis* is dominant species of majority of regions. However further studies are necessary to explain why *T. vassilievi* or other species precede *T. grandis* some years in some regions.

Keywords: Searching efficiency, egg parasitoid, interference

CO069

DISRUPTION OF LOCAL CHEMICAL COMMUNICATION SYSTEMS BY THE INVASIVE INSECT HERBIVORE, *HALYOMORPHA HALYS*

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Established invasive herbivore insect species can compete with native species on the same host plants by altering the plant volatiles used as cues for the herbivores, their natural enemies, and pollinators, ultimately leading to irreversible changes on the local biodiversity. The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Heteroptera: Pentatomidae) is a species native to Asia, which was recently introduced in the Old and New World where it can share host plants with local pentatomid pests. Previous studies demonstrated that in the tritrophic systems consisting of vegetable plants, hemipteran true bugs and their scelionid egg parasitoids, the wasps are attracted by oviposition-induced plant volatiles (OIPVs). To evaluate the potential impact of the BMSB on local infochemical networks, laboratory experiments were conducted using two systems, one from Europe, consisting of *Vicia faba - Nezara viridula - Trissolcus basalis*, and the other system from North America, represented by *V. faba - Podisus maculiventris Telenomus podisi*. Both egg parasitoid species, *T. basalis* and *T. podisi*, were attracted by the OIPVs of their associated hosts, *N. viridula* and *P. maculiventris*, respectively, but not by the OIPVs of the BMSB. However, in both systems, the parasitoid attraction toward OIPVs emitted following the attack of the associated host was disrupted when host and non-associated host were concurrently present on the same plant. This indicates that invasion by an alien herbivore can interfere with local established semiochemical webs.

Keywords: Oviposition-induced plant volatiles, *Halyomorpha halys*, Insect invasion, Multiple attack, *Trissolcus basalis*, *Telenomus podisi*

CO070

FIELD TESTS OF MULTIPLE SENSORY CUES IN SEX RECOGNITION AND HARASSMENT OF A COLOUR POLYMORPHIC DAMSELFLY

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Odonates have long been assumed to use only visual cues in recognizing potential mates. We previously demonstrated, in the lab, that males of the damselfly *Ischnura elegans* are attracted by their polymorphic mates and can discriminate between the sexes and female color morphs using only air-born cues. In the present research we tested how free-flying males under field conditions reacted to conspecifics in the absence of visual cues. ‘Non-visual’ treatments consisted of live individuals of a given type (male or females of the different morphs) attached to dowels, or empty control dowels, concealed under a muslin bag placed in an area close to the pond water. ‘Visual’ (plus odor) treatments were the same uncovered dowel sets, placed in the same area adjacent to water. Male reactions during 10 min were scored as non-sexual or sexual. In the non-visual treatments, male reactions towards live conspecifics and control did not differ. In the visual treatments, male sexual responses were higher towards live conspecifics (both males and females) compared to controls, but male sexual reactions were quite low. We also quantified natural male harassment rates of marked, free-flying females. We found no difference in harassment or mating rate, as well as behavior to evade males, between female color morphs. We discuss the relevance of our findings considering how the use of multiple sensory modalities in mating decision can influence sexual selection and maintenance of female polymorphism in Odonata and more generally.

Keywords: Coenagrionidae, *Ischnura elegans*, multimodal signaling, Odonata, olfaction, sexual behaviour, sexual conflict

CO071

I WILL WAIT: THE STRIKINGLY LONG MATING-RELUCTANCE PERIOD OF A BRACONID PARASITIC WASP

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The gregarious ectoparasitoid *Bracon brevicornis* (Wesmael) has complementary sex determination (CSD). Especially under inbreeding conditions, CSD leads to the production of diploid males at the expense of fertile daughters. Diploid males in this species are able to sire triploid daughters at a low rate, which only occasionally produce offspring. Avoidance of sib-mating should reduce costly diploid male production, and has been found in several Hymenoptera with CSD. In B. brevicornis, however, we observed a frequent occurrence of sib-mating, as brothers were almost as often accepted as mates as were less related males. From a series of experiments we conclude that a post-hatching mating reluctance, which lasts 24-48 hours in this species, might facilitate the dispersal of virgin females from the natal patch, and thus lead to a high probability of mating with a random male from the population, instead of a brother. We analyzed the duration of the mating-reluctance period in two different wasp populations, and across two generations, to look for (genetically based) variability in this behaviour. We also tested whether very young females that rejected the male would have been able to physically mate successfully. The results are put into context and discussed along with other more common mechanisms of inbreeding avoidance in parasitoid wasps.

Keywords: Mate choice, inbreeding depression, complementary sex determination, Braconidae, dispersal

CO072

PARASITOIDS BEHAVIOR AND EFFICIENT BIOLOGICAL CONTROL

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Parasitoids are fascinating insects that are using elaborated reproductive strategies to find and to attack their hosts in an efficient way in their foraging environment. For this reason, these insects have served as model species to develop a rich corpus of theory with the aim to understand what sort of behaviours they have developed in the course of the evolutionary time to maximize the number of progeny that can produce during their adult life time. Moreover, some of these insects are produced and released in the field to control noxious phytophagous pests though so-called biological control programs. The efficacy of such insects to control and kill their hosts, and thus their efficacy as biological control agents, is actually based on their ability to find and to attack their hosts, which is obviously determined by the foraging behaviour they are adopting. The talk will present the main behavioural components of parasitoid insects on which the scientific community worked the last decades. In all cases, the way the results obtained can be used to enhance their pest control efficacy will be discussed.

Keywords: Parasitoids, Behaviour, Behavioural Ecology, Biological Control

Session 3.
Biological Control and Integrated Pest Management

Breeding Invertebrates for Next Generation Biocontrol

CO073

SELECTIVE BREEDING OF THE BIOLOGICAL CONTROL AGENT *ORIUS LAEVIGATUS*: BIOTIC POTENTIAL AND TOLERANCE TO ENVIRONMENTAL STRESS

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Augmentative biological control has proven to be an efficient and robust pest control method. However, there are serious limitations in their implementation in some crops, areas and/or periods of the year, which compromise their effectiveness throughout the crop cycle and make it difficult to expand to other crops or agricultural areas. There are limitations to the establishment and success of protocols of biological control by environmental conditions, adaptation to crops, compatibility with pesticides, etc. It is not always possible to find new species of natural enemies that are better adapted, or even the importation of exotic species can lead to other problems. Species of natural enemies are adapted to their natural environment through natural selection. However, the conditions under which biological control agents must exercise their beneficial action (crops, greenhouses) are often very different from natural ones. Therefore, the optimal characteristics for their performance in the different agro-systems must be selected through artificial selection. Therefore, a real possibility of progress in biological control is the genetic improvement of biological control agents to respond to present and future challenges. As has happened in the evolution of agriculture with plant varieties and animal breeds, the artificial selection of biological control agents with certain characteristics can contribute decisively to a greater success of biological control. In this talk we will discuss the most recent results of our genetic improvement program of one of the main biological control agents used in augmentative biological control, *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae).

Keywords: Orius, Anthocoridae, predator, thrips, insecticide resistance, fecundity

CO074

TRENDS IN BIOLOGICAL CONTROL

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Biological control has secured a position among the most sustainable and effective approaches to pest control. After decades of practice in modern pest management, it is appropriate to investigate trends in biological control, both to capture its evolution and to explore future opportunities. In this seminar, I aim to examine aspects of (i) international networking, (ii) research in biological control and (iii) public interest to identify trends and their change over recent time. Biological control has a prominent international profile characterized by multilateral collaborations. This facet has increased over the last 25 years and now defines the practice of biological control more than ever. Biological control has become a thriving scientific discipline with several major research trends in arthropod, plant pathogen and weed management. This multidisciplinary field is grounded in ecological and evolutionary principles but also embraces environmental, economic and social considerations. However, a Google Trends analysis revealed that the popularity of biological control is decreasing in terms of search hits on the internet. This trend is partly due to the damaging consequences of the ongoing debate on non-target impacts, but other interrelated factors are obviously involved.

Keywords: Biological control

CO075

TRICHOGRAMMA POPULATIONS IN ORGANIC GERMAN CABBAGE FIELDS – A GENETIC, ECOLOGICAL, AND BEHAVIOURAL OVERVIEW

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Since 1985 egg-parasitoid wasps of the *Trichogramma* genus have been the most released entomophagous arthropods for biological control practices across the globe. Extensive non-target risk assessments have been carried out in recent years, however the intraguild effect of such mass-releases on the naturally occurring Trichogramma populations, as well as the parasitoid communities as a whole, have been largely overlooked. Therefore, over the summer of 2017 a total of 18 cabbage fields on 15 organic farms in six distinct areas on a North – South cline of Germany were sampled weekly for pest and parasitoid presence. Emergence of Trichogrammatid wasps from collected Lepidopteran eggs, as well as the presence of larval- and pupal parasitoids, was recorded weekly for every field, giving an impression of natural parasitism rates and parasitoid presence in organic cabbage fields in Germany. Trichogramma populations were ID'd to species level using ITS2 and CO1 mitochondrial sequences via the Rotorgene technique, with the majority of species found to be Trichogramma evanescens. Microsatellite markers were used to give an estimate of the relatedness of Trichogramma populations within and between areas on the North – South cline of organic cabbage fields in Germany. Life history trait analyses, including longevity, sex ratio, fecundity, and mortality, were carried out at different temperatures in order to determine population differences along the cline. Additional behavioural assays comparing the effect of intra- and interspecific competition on the different populations showed clear differences both between the different species as well as the distinct populations. This current study provides important genetic, ecological, and behavioural information on the parasitoid, in particular Trichogramma, communities already present in organic German cabbage fields, important to keep in mind for biocontrol release risk-assessment studies.

Keywords: Trichogramma, parasitoid, species distribution, biocontrol, population genetics, life history traits

CO076

IS VEGETARIANISM IN THE GENES? INVESTIGATING THE GENETIC BASIS OF PHYTOPHAGY IN *NESIDIOCORIS TENUIS*

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Artificial selection towards the improvement of certain traits has been a common practice in mammals. However, selection attempts in arthropods have been limited to only a few species due in part to the lack of knowledge on the genetic basis for complex traits on which selection can be applied upon. Foraging behavior is one of those complex traits with an important potential for exploitation in biological control. The use of zoophytophagous predators for pest control in cultivated systems in Europe has peaked in the last decade, mainly due to the ability of these species to feed on plant tissue and remain in the crops even before the pest arrives or when pest populations are low. Nevertheless, under certain circumstances this phytophagous behavior can lead to plant damage. *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) is one of those zoophytophagous species which foraging behavior could be improved through artificial selection. For this, we decided to evaluate the intraspecific genetic variation for phytophagy in a wild population of *N. tenuis* to determine whether artificial selection is a viable approach. Wild specimens were collected and brought to the laboratory to establish a colony. Ten isofemale lines were created by mating one virgin female and one male and allowing the offspring to reproduce for eleven generations without generational overlapping. Phytophagy was assessed by placing ten adult individuals on a tomato leaf enclosed in a muslin bag without prey and allowing them to feed on the plant tissue for 5 days. After this time period insects were removed and plant damage measured as number of necrotic rings and percentage of wilted leaf surface, was evaluated. Our results can serve as a basis for the artificial selection of biological control agents based on specific traits susceptible of selection.

Keywords: Zoophytophagy, genetic variation, biological control, predator

CO077

AN INCREASED NEED FOR RESEARCH ON BIOLOGICAL CONTROL

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The global agriculture is faced with a challenge: productivity needs to increase to feed the growing population while at the same time the reliance on chemical crop protection needs to be reduced to increase the sustainability. This can be resolved by the implementation of biological control. The use of biological crop protection has increased by 15% per year in the past years and this growth is expected to continue, or even accelerate in the years to come. To facilitate this growth, more research on biological control is required. While the biocontrol industry is currently investing in its own research capacity, collaboration with academic researchers also remains essential. I call for an increase in this collaboration on a large range of different topics. There is a gap between academic science and the need for scientific knowledge from farmers. Too often, academic research is unaware of this gap. To close it, I like to challenge the entomologists in the academia to consider how their science can help farmers to grow their crops more sustainably. Reaching out to solve practical problems might not result in high-impact research papers that are required to pursue an academic career. Therefore other systems may be required to appreciate this type of scientific research.

Keywords: biological control, agriculture, sustainability

CO078

USING GENETICS TO IMPROVE BIOLOGICAL CONTROL

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Secure and sustainable food production in terms of quantity and quality is a major challenge facing human societies. However, food security is continuously threatened by current and invasive pest species. In addition, regulations for the use of pesticides are getting stricter to ensure food safety and protect ecosystem health. Biocontrol of agricultural pests by using natural enemies has great potential to deal with these two demands. Biocontrol of novel exotic pests, however, often involves importing non-native natural enemies. Such practices are being restricted due to the Nagoya Access and Benefit Sharing regulations, and to potential risks to local biodiversity. The dependence on imported natural enemies can be reduced by optimizing existing and native biocontrol agents. Within the BINGO (Breeding Invertebrates for Next Generation BioControl) Training Network, academia, institutions and industry join forces to advance current knowledge in biocontrol practice through the use of natural genetic variation. In 13 interlinked research projects, we aim to improve the efficiency of biological pest control through selective breeding of natural enemies in a broad range of agricultural systems and environmental conditions. These projects address current bottlenecks in biocontrol, for rearing, monitoring and performance using a broad range of scientific disciplines, including agro-ecology, modelling and population genetics and genomics. In this presentation, I will present the BINGO network, and highlight some of our recent results on the use of genetic knowledge for the improvement of biological control.

Keywords: Biological control, biological control agents, genetics, genomics, genetic improvement, optimization

CO079

EFFECT OF MASS REARING ON THE PERFORMANCE AND THE GENETIC DIVERSITY OF THE PREDATORY MITE *AMBLYSEIUS SWIRSKII* ATHIAS-HENRIOT (ACARI: PHYTOSEIIDAE)

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Amblyseius swirskii is a predatory mite used to control several pests in protected crops. This biological control agent (BCA), firstly collected in Israel, has been mass-reared for commercial use since 2005 and nowadays it is widely used in augmentative biocontrol programs. As a polyphagous predator, it has to cope with different biotic and abiotic factors. However, the adaptation to mass rearing might be hindering its resilience and capacity for optimum performance. In this study, we investigated the effect of long-term adaptation to mass rearing on the performance and genetic diversity of *A. swirskii*. We assessed the predation and oviposition capacities of a wild population and a commercial strain of *A. swirskii*. Furthermore, we identified 6 microsatellite (ms) loci across the genome of *A. swirskii* and 8 wild populations collected from Israel were scored for those ms markers. Average heterozygosity (H), average allelic diversity (A) and the number of private alleles (Np) and pairwise Fst among populations were calculated. Also, pairwise genetic distances between populations were used in Neighbour Joining (NJ) analysis and Multi-Dimensional Scaling. Our results indicate that predation rate on *Ephestia kuehniella* eggs was lower in the commercially reared *A. swirskii* strain when compared to wild *A. swirskii*. Similarly, the commercial strain has only 39.7% of the heterozygosity which is present in wild *A. swirskii*. On the other hand, the commercial strain has the highest genetic differentiation from all the natural populations, as indicated by the pairwise Fst values. The clustering analysis suggests a possible effect of the plant on the genetic structure of the mites. Overall, we have showed that commercial BCA may have reduced genetic variation compared to their wild counterparts, which may reduce their performance when released to control herbivores in an IPM context.

Keywords: biological control, population genetics, microsatellites, genetic differentiation

CO080

INTRINSIC COMPETITION BETWEEN TWO EUROPEAN EGG PARASITOIDS OF THE BROWN MARMORATED STINK BUG

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The brown marmorated stink bug, *Halyomorpha halys*, is invasive in Europe and causes significant damage in economically important crops. *Anastatus bifasciatus* and *Ooencyrtus telenomicida* are the only known European parasitoids capable of killing *H. halys* eggs and thus have been considered for inundative biocontrol. Multiparasitism of eggs can occur, but the outcome of larval competition is poorly understood and may influence the success of biocontrol. Naïve females of *A. bifasciatus* were allowed to parasitize *H. halys* eggs, which were then presented to *O. telenomicida* 0, 1, 2, 3, 4, 5, 9 or 14 days later. The same protocol was used with the parasitization order reversed. Multiparasitized eggs were reared and offspring was identified to species. For *A. bifasciatus* there was no significant difference between acceptance of eggs containing different stages of *O. telenomicida* and unparasitized controls, but from day nine onwards eggs containing pre-pupae and pupae of *O. telenomicida* were parasitized less. *O. telenomicida* females showed a similar behavior, but parasitism decreased from day four onwards, when eggs contained mid-size larvae of the competitor. In general, both species were able to develop on multiparasitized eggs. Independent of the order of parasitization, the proportion of *A. bifasciatus* emerging from multiparasitized eggs was generally higher than that of *O. telenomicida*. This indicates that larval competition influences *O. telenomicida* negatively and may lead to less offspring overall when *A. bifasciatus* attacks already parasitized eggs. For inundative biocontrol, no negative impact should be expected.

Keywords: Biological control, *Halyomorpha halys*, larval competition, inundative biocontrol, *Anastatus bifasciatus*, *Ooencyrtus telenomicida*

CO081

IMPROVING THE GENETICS OF NATURAL ENEMIES FOR BIOLOGICAL CONTROL WITH LESSONS NOT YET LEARNED FROM THE LAST CENTURY

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Prolonged rearing biocontrol agents is an unavoidable part of classical and augmentative biological control projects. Yet, little practical attention has been given to the potentially very negative impacts of such prolonged rearing for the effectiveness of biocontrol agents once they are released in the field. Several effects are known of prolonged mass rearing and these include: loss of genetic variation and adaptation to mass rearing conditions (domestication). In classical biological control the release of natural enemies lacking genetic variation can result in either the establishment of populations that are ineffective, or complete failure of establishment. Recently it has been shown that introduction of natural enemies that lack genetic variation can also lead to selection for resistance in the host. Problems caused by this lack of variation in classical biological control cannot be solved by releasing more individuals of the same mass reared population, since they will also lack genetic variation. In augmentative biological control where the released natural enemies have to do the control without the expectation that they will establish long term populations, quantity can compensate for a lack of quality. However, also for augmentative biological control release of natural enemies that have not been selected for mass rearing conditions is expected to result in better control. Fortunately, both in classical biological control and in augmentative biocontrol the problems associated with lack of genetic variation and domestication of the natural enemies can be avoided by simple rearing measures that are not very expensive. These include maintaining separate populations, a large number of isofemale lines etc.

Keywords: Biological control, genetics, mass rearing

Habitat and Landscape Management to Improve Conservation Biological Control

CO082

EFFECT OF FIELD MARGIN ELEMENT TYPE ON HOVERFLY ABUNDANCE

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Field margins have the potential to provide resources to beneficial insects in disturbed environments such as agroecosystems. In 2016 and 2017, a study was performed in an organic farm to investigate the effect that different field margin element types had on adult hoverfly abundance, a family that contain many species that provide ecosystem services. Five different type of field margin elements were identified; trees, shrubs, ditches, grass margins, and tracks. Generalized Linear Mixed Models were used to analyse the relationship between syrphid abundance and field margin element type as well as other factors. Field margin element type and the calculated flower index for each sampled field margin were found to significantly affect syrphid abundance. A higher abundance of syrphids were recorded in ditches, trees, and shrubs than in cropped fields. The effect size of grass strips and tracks on syrphid abundance was not significantly larger than the calculated effect size of fields. A positive effect of flower index on syrphid abundance was also detected by the model. Although tracks had one of the lowest effect size, they provided more flower resources than any other element type. This suggests that the importance of element type in attracting hoverflies is not just based on their potential in offering abundant flower resources but also on their ability to offer other resources such as ovipostioning sites or shelter.

Keywords: Agroecology, beneficial organisms, natural predators, pollinators, semi-natural habitats, syrphidae

CO083

SOIL LEGACY EFFECTS ON PLANTS AND THEIR ASSOCIATED ABOVEGROUND INSECT HERBIVORES ARE LINKED VIA PLANT FUNCTIONAL AND NUTRITIONAL TRAITS

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Plants affect the living environment in their rhizosphere. These biotic effects are often plant-species specific and can persist in the soil after the plant disappears. Such soil legacy effects can, in turn, have strong effects on plants that grow subsequently in the same soil (plant-soil feedback, PSF), but also the associated aboveground insect herbivores. Mechanisms have not been adequately described, but it is likely that growth and defense traits play a role. We hypothesized that plants that suffer more negative soil legacy effects would be poorly defended and thus would be vulnerable to herbivores. We further expected that nutritional and functional traits of the plant would be related to how plants and aboveground insects respond to species-specific legacy effects in the soil. Using twelve common grass and forb species that differ in growth rate we examined the relationship between soil legacy effects and the performance of aboveground herbivore *Mamestra brassicae* on these plants. We found a positive correlation between the strength of both conspecific and heterospecific PSF and the performance of herbivores on the plant species. Interestingly, the effect in the latter was driven mostly by forbs, whereas in the former, the relationship was observed for grasses and forbs. There was a negative relationship between nutritional characteristics of a plant species and the effect that the plant species has, via influencing the soil, on future herbivores on plant species that grow in the conditioned soil. We show positive relationships between both conspecific and heterospecific PSF effects of a plant species and the effects that their soils have on the performance of a future herbivore. We argue that more vulnerable species (either more nutritious or poorly defended species)can recruit beneficial soil microbes in their soils, which may help them in their defenses against herbivores in the future (or future generations).

Keywords: Herbivory, plant-soil feedback, traits, defense, soil legacy effects, microbes

CO084

STACKING CROP VARIETIES AND INTERCROPPING IMPACTS APHIDS IN WHEAT FIELDS

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Increasing within field plant diversity has been shown to regulate pest populations in various agroecosystems. Diversification practices at the field level might concern intraspecific diversity as polyvarietal mixtures of a crop species or interspecific diversity such as two crops (intercropping) or a crop and a beneficial non-crop plant (cover cropping or living mulches). Both practices are considered as promising agroecological practices for low-input or organic agriculture by providing several ecosystem services such as diseases control, weed control and N fertilization. However, combining both diversification practices has not been studied yet in perspective of winter wheat pest control. We examined the impact of a mixture of two wheat varieties and a white clover - winter wheat intercrop on aphid populations in nine field experiments settled on organic farms in South-East of France over two growing seasons. From March to June 2016 and 2017, aphid populations were monitored on wheat tillers and crop canopy characteristics were recorded (plant heights and percentage of soil cover) every two weeks. Grain yield and N content was measured at harvest to determine the impact on production service. Mixing wheat varieties had a positive effect in reducing the overall aphid population in 2016 but not in 2017. Inversely, intercropping white clover reduced the overall aphid population only in 2017, while clover had difficulties to establish this year. Aphid peaks occurred at different wheat growth stages in 2016 (before flowering) and in 2017 (after flowering). A synergistic effect between diversity levels was observed in 2016 but the mean sum of aphids was not different from the most resistant wheat variety in monoculture. Wheat yields were reduced by about 10% and grain N content by 5 to 8% due to the intercropping with white clover. Interspecific competition might be the main responsible for these observed effects.

Keywords: Biological control, Mixtures of varieties, Intraspecific diversity, Cover crop, Interspecific diversity, Organic agriculture

CO085

SEEKING MULTIPLE ECOSYSTEM SERVICES FOR AGRICULTURE FROM NATIVE AUSTRALIAN PLANTS

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Ecosystems provide humans with a range of services that comprise provisioning, regulating, supporting and cultural services. However, efforts to increase provisioning services such as food and fiber production can compromise important regulating ecosystem services that underpin the sustainability of agroecosystems. Habitat management modifies agroecosystems by tactics such as planting companion plants to benefit natural enemies for conservation biological control. But this added plant diversity might also provide additional ecosystem services. Here we highlight results from studies in which Australian native plants were examined for beneficial effects on natural enemies through enhancement of longevity and measurements were also made of the strength of other ecosystem services. We found eight of twelve native Australian plants studied in the laboratory significantly enhanced the longevity of one or more parasitoids (*Diaeretiella rapae* and *Cotesia glomerata* (Hymenoptera: Braconidae), and *Diadegma semiclausum* (Hymenoptera: Ichneumonidae) when floral shoots of them were presented. On-farm work found significantly higher abundance of natural enemies (both ground dwelling and canopy dwelling) in areas of crop that were close to perennial native vegetation. This effect was, however, masked when crops were heavily sprayed with insecticides. A third study used a designed experiment to measure multiple ecosystem services: pollinator enhancement, soil biological activity and conservation biological control from three Australian natives and one non-native plant species. Results will be discussed in relation to the scope to minimize trade-offs and deliver additive or even synergistic effects among multiple ecosystem services.

Keywords: Ecosystem services, habitat management, native plants

CO086

INFLUENCE OF A REFUGE AND SUGAR SOURCES ON *APHELINUS MALI* TO CONTROL *ERIOSOMA LANIGERUM* IN APPLE ORCHARDS

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Conservation biological control practices could be an alternative to the actual apple orchard pest management strategies, by increasing the accessibility of food sources and refuge for natural enemies that are often in short supplies. Parasitoid persistence and reproduction are limited by the availability of these two elements and neither the presence of both separately ensures the increase in parasitism of the pest; however, their combination could increase parasitoid efficiency in regulating pest populations. In the present study, we assess the effects of *Pyracantha coccinea* as a refuge for parasitoids and of aphid honeydew in apple orchards to control *Eriosoma lanigerum* by *Aphelinus mali*. Field bioassays and population dynamics were carried out for the pest and its parasitoid from different distances to *P. coccinea* during two seasons. Additionally, the influence on the

behaviour of *A. mali* in the presence of different sugars sources was examined conducting laboratory experiments. Results indicate that *P. coccinea* could have an early effect on the parasitism by *A. mali*. However, as time pass the parasitism increases in the center of the field showing significantly higher rates of parastism at the end of the season compared to apple plants close to the *P. coccinea*. In addition, although *A. mali* consumes honeydew excreted by *E. lanigerum*, the effect of this sugar on the frequency of parasitizing the aphid does not increase significantly. The foraging time of *A. mali* with its host increases significantly in the presence of aphid honeydew, which could indicate that some compounds in this sugar are acting as a host-location kairomone rather than as a food source for *A. mali*. Given these findings, the possibility of linking the conservation and inundative biological control strategies should be considered to increase the efficiency of *A. mali* in controlling *E. lanigerum* in apple orchards.

Keywords: Conservation biological control, refuge, honeydew, parasitoid, aphid, apple orchard

CO087

THE MANAGEMENT OF PLANT BIODIVERSITY IN FIELD MARGINS FOR THE ENHANCEMENT OF NATURAL BIOLOGICAL CONTROL

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Agriculture has greatly shaped the landscape on earth, but its impact on the earth's natural processes has reached an outrageous magnitude over the last century. In many places the intensification of agriculture has led to the implantation of monocultures and the reduction of the extension of natural ecosystems. Such decline in biodiversity is associated with a reduction in ecosystem services that are generally supplied by external inputs. Nowadays, we face the great challenge of maintaining/increasing crop productivity through sustainable strategies of low environmental impact based on the management of biodiversity. The aim of this work was to test how edges of vegetation affected the community of arthropods in melon crops and to identify the plant species that serve as hosts for key natural enemies. The work was conducted in four farms located in south-eastern Spain during 2014. In each farm, three types of margins (shrubby, herbaceous and non-revegetated) were assayed in melon crops. A mix of plants from different families (*i.e.* Apiaceae, Asteraceae, Boraginaceae, Brassicaceae, Caryophyllaceae, Fabaceae and Lamiaceae) was used in shrubby and herbaceous edges; in non-revegetated margins, the wild vegetation was left to flourish naturally. The margins and melon crops were sampled periodically from February to July to determine the abundance of arthropods. Shrubby and herbaceous edges of vegetation had a significant effect on the community of arthropods in melon crops. For instance, the abundance of *Bemisia tabaci* and *Tetranychus spp.* in melon were significantly influenced by the type of field margin, and *Orius spp.* was significantly more abundant in melon crops with rather than without revegetated margins. Plant species in margins differed qualitatively and quantitatively in the phytophagous and natural enemy species they hosted. A proper margin design is needed for the enhancement of the most desirable aspect of biodiversity in each particular case.

Keywords: Biodiversity, Ecosystem services, Edges of vegetation, Melon, Predators, Pests

CO088

FUNCTIONAL AGROBIODIVERSITY IN APPLE ORCHARDS

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Fruit growers often suffer economic losses due to insect damages. The available natural pest control products are not always effective; and for organic fruit growers options for direct control are relatively few. EcoOrchard, a CORE Organic Plus project (2015-18) tracked EU farmers knowledge of Functional AgroBiodiversity (FAB) and generated new knowledge in order to successfully use FAB in orchards by a joint field experiment across seven countries of perennial flower strips to assess impact on natural enemies, codling moths and yield. The project also established the EBIO-Network as a European-wide network of stakeholders for collecting, sharing and improving scientific and practical knowledge and experience in FAB management, and developed simple FAB assessment tools for on-farm use by growers, so that they can better understand FAB effects in their own orchard; finally, partners used a participatory approach to learn about potential constraints that may hamper the adoption of innovative tools and how to solve these constraints by iterative reevaluation. Prevention of pest damage can reduce the need for direct control measures and the positive effect of floral diversification on natural enemy abundance and diversity is well documented also from orchards. Our review shows that fewer studies have linked this to effects on pests, and even fewer still to effects on yield, though this is the ultimate test seen from a production perspective. Still the few existing studies point to a positive effect on yield, and hereby corroborate our results linking increased abundance of natural enemies with reduced loss due to insect pests. Farmers already implement several techniques to favor FAB at different scales of their farm but have little knowledge and expectations about their benefit, and techniques for design, management and assessment tools such as the FAB-handbook provided, can further support its adoption and development.

Keywords: Functional agrobiodiversity, apple orchard, predator, yield, *Dysaphis plantaginea*, *Cydia pomonella*

CO089

HABITAT AND LANDSCAPE MANAGEMENT TO IMPROVE CONSERVATION BIOLOGICAL CONTROL

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Management of biodiversity services such as biological control in agriculture needs to overcome a spatial mismatch, as farmers are interested in local-scale management of their field or farm, whereas success depends mainly on improvements at the landscape scale. The dependency of local autonomous biological control in cropland on the surrounding landscape structure is often overlooked. For example, simplifying landscapes, *i.e.* eliminating all near-natural habitat patches in favor of only annual crop fields, leads to high oilseed rape damage and low parasitism of rape pollen beetles, even in conventionally managed fields. Similarly, control of cereal aphids is higher in complex compared to simple landscapes. Success of local management, *e.g.* of weeds and field boundaries, as well as success of landscape management, *e.g.* of landscape configuration, changes with traits of pests and their enemies, such as body size, dispersal ability and specialization. In the tropics, pest-enemy interactions can be complex, while still providing highly valuable control. In cacao agroforestry, ants have been shown to provide major services, including reduced leaf and fruit herbivory and indirect pollination facilitation, but also disservices, such as increased mealybug density, phytopathogen dissemination and indirect pest damage enhancement. Altogether, cacao yields were higher with species-rich and even ant communities. Similarly, exclusion of birds and bats were shown to reduce cacao yield by a third. These and other results highlight the often tremendous economic impact of common natural enemies, which needs to become an essential part of future research and sustainable landscape management.

Keywords: Scale mismatch, landscape configuration, temperate and tropical agriculture, traits

Exotic and Native Natural Enemies: How to Plan Biological Control and Save Environments

CO090

INVESTIGATING THE FUNCTIONAL RESPONSE OF TWO ANTHOCORID PREDATORS TO *TETRANYCHUS URTICAE* (ACARI: TETRANYCHIDAE) AND *BEMISIA TABACI* (HEMIPTERA: ALEYRODIDAE)

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Orius spp. is well-known as a generalist predators' genus, preying upon numerous pest insects and acaries in cropping systems. In this study, functional response of the anthocorid predators, *Orius laevigatus* (Fieber) and *Orius vicinus* (Ribaut) (Hemiptera: Anthocoridae), to two economically important pests' eggs, namely *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and *Tetranychus urticae* Koch (Acari: Tetranychidae), were investigated under the laboratory conditions. Different densities as 2, 4, 6, 8, 16, 32, 64 and 128 eggs were offered to the female predators for 24 hours period under conditions of 25±1°C, 60±10% RH and 16:8 h (L:D). The parameters of functional response were assessed by using the Holling Disc Equation. Both predators showed the Type II response to both prey. According to estimations, the attack rates and handling times for predators were computed in the way that to whiteflies eggs: *O. laevigatus* (a: 1.022, Th: 0.006) and *O. vicinus* (a: 0.772 Th: 0.002); to spider mites eggs: *O. laevigatus* (a: 0.972, Th: 0.005) and *O. vicinus* (a: 1.113, Th: 0.007). Furthermore, the average of the consumed *B. tabaci* eggs by *O. laevigatus* females was greater than *O. vicinus*. On the contrary, *O. vicinus* exhibited more efficient predation on *T. urticae* eggs than *O. laevigatus*. Consequently, these hopeful results may suggest that both predators could be effective biological control agent in regulating the *B. tabaci* and *T. urticae* in agricultural ecosystems.

Keywords: Functional response, biological control, *Orius vicinus*, *Orius laevigatus*, *Tetranychus urticae*, *Bemisia tabaci*

CO091

AUGMENTATIVE RELEASES OF *TRICHOPRIA DROSOPHILAE* FOR THE CONTROL OF EARLY-SEASON *DROSOPHILA SUZUKII* POPULATIONS

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Biological control agents may play an important role in regulating *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), spotted wing drosophila (SWD), particularly after the winter bottleneck of the population. In this work we tested the ability of the cosmopolitan pupal parasitoid, *Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae), of reducing early SWD populations in order to increase the efficacy of other control techniques throughout the season. We selected four similar sites located in Trento Province (North-Eastern Italy), each one hosting a single cherry orchard and several isolated cherry trees. During late March-April, we performed augmentative releases of the parasitoid in two of these sites, while leaving the others untreated. We carried out an extensive monitoring activity on both parasitoids and flies populations. Moreover, we evaluated SWD infestation and eclosions from plant-sampled and ground-sampled fruit respectively. Results clearly showed a mitigation of the SWD population in the treated areas, associated with a higher *T. drosophilae* parasitism. Both the fruit infestation and the SWD eclosions were significantly reduced in the isolated cherry trees surrounding the crops in the treated areas, while no difference was observed for the fruit sampled in the cherry orchards. Our results open to the possibility of using *T. drosophilae* as a low-cost and sustainable tool for taking action on SWD populations in the unmanaged areas surrounding the crops.

Keywords: Biological control, spotted-wing drosophila, Diapriidae, Field trials, parasitism, Cherry

CO092

FIELD DISPERSAL AND FIELD PERSISTENCE OF THE PARASITOID WASP *HABROBRACON HEBETOR* (HYMENOPTERA: BRACONIDAE) FOLLOWING AUGMENTATIVE RELEASES AGAINST THE MILLET HEAD MINER *HELIOCHEILUS ALBIPUNCTELLA* (LEPIDOPTERA: NOCTUIDAE) IN THE SAHEL

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Biological control by augmentative releases of the parasitoid wasp *Habrobracon hebetor* Say (Hymenoptera: Braconidae) is the most promising strategy for controlling the millet-head miner (MHM) *Heliocheilus albipunctella* (de Joannis) (Lepidoptera: Noctuidae). The current biological control program encourages fresh releases of the parasitoid in each growing season with no detail on releases distances. We studied the residual effects on the parasitism of the MHM years after augmentative releases of parasitoids. We also investigated the effect of two successive annual augmentative releases of *H. hebetor* on the parasitism level of the MHM. We also studied the dispersion patterns of the parasitoid, after augmentative releases through weekly assessments of MHM parasitism by *H. hebetor* at different distances from release points (0, 3 and 5 km) and in control villages (15

km). Our findings on field persistence indicate that two successive releases of parasitoid did not increase the parasitism of the MHM. However, after an initial release of parasitoids, the parasitism level decreased in subsequent years if no additional parasitoids were released. Nevertheless, in the first year after releasing the parasitoid, the parasitism level remained significantly higher than that in control villages, which did not receive any parasitoids. Regarding field dispersal, the initial parasitoid population released in the environment led to higher parasitism of MHM at the site of dissemination compared to farms at distances of 3 and 5 km. However, usually after 5 weeks, successive generations of *H. hebetor* dispersed up to 3 km, causing high levels of MHM larval mortality, which sometime are similar to those of the release points. These findings suggest that augmentative releases of parasitoid could be carried out every 2 years instead of the current annual releases at sites spaced 3 km for timely and more efficient control of MHM populations.

Keywords: Biological control; parasitoid bag; parasitoid survival; on-farm releases; *Corcyra cephalonica*; Burkina Faso; Niger

CO093

TACHINID PARASITOIDS AS EXOTIC OR NATIVE NATURAL ENEMIES OF HERBIVOROUS INSECTS

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The role of tachinid parasitoids as natural enemies of herbivorous insects is underestimated. This is partially due to their relatively low number, as they represent fewer than 20% of all insect parasitoids, most of which are hymenopterans. Yet, this family of dipterans is the largest and most important group of non-hymenopteran parasitoids, due to their characteristics, which include different oviposition strategies that allow them to parasitize hosts in different environments. Many tachinids are unique because of their capacity to attack hosts hidden in the soil or in plants. Although these parasitoids are overall disregarded, there are several examples of attempts (either successful or not) to utilize them as exotic natural enemies of insect pests in classical biocontrol programs, especially in the Nearctic and Neotropical regions and, to a lesser extent, in the Australian region. Conversely, examples in the Palearctic region are lacking. Failures were often due lack of knowledge on parasitoid biology and host-parasitoid interactions, as well as improper rearing procedures and shipment methods. The use of native tachinids in augmentative biocontrol programs against herbivorous insects has been even rarer. To date, a very few species (all natural enemies of lepidopterans) have been mass produced in biofactories, mainly in Central and Latin America. In Europe, the potential of tachinid parasitoids as biological control agents deserves to be better investigated. Some native, or naturalized, species (*i.e. Exorista larvarum* and *Trichopoda pennipes*) have shown the potential to adapt to exotic pests, such as, for *E. larvarum*, the geranium bronze *Cacyreus marshalli* and the box tree moth *Cydalima perspectalis* and, for *T. pennipes*, the brown marmorated stink bug *Halyomorpha halys*. Studies on acceptance and suitability of these exotic hosts for the two tachinid species may constitute a base for their exploitation as natural enemies of the target insect pests.

Keywords: Tachinidae, native and exotic species, biological control

CO094

THE ASIAN CHESTNUT GALL WASP: THE SUCCESSFUL MANAGEMENT OF AN EXOTIC INVASIVE PEST

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In the last decades the number of invasive alien species in Europe has increased significantly and is considered as a major cause of serious economic and biodiversity loss. In the literature several examples of invasive insect pests accidentally introduced through global trade and travel are available. At the beginning of the XXI century the Asian chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), has been reported for the first time in Europe (Italy), and rapidly spread to many other European countries. In order to suppress the gall wasp population growth, the parasitoid *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) was released. A classical biological control program was initiated in 2005 with the release of Japan-imported *T. sinensis* specimens in infested chestnut orchards. A long-term monitoring was carried out in Italy in a nine-year period in different chestnut-growing areas. A quantitative assessment of the effectiveness of this parasitoid was provided, evaluating the trend both of the infestation rate by *D. kuriphilus*, and of the parasitism rate by *T. sinensis*. After its introduction, *T. sinensis* caused a density-dependent mortality on *D. kuriphilus*, and in a few years reduced considerably the population density of the gall wasp, making this parasitoid one of the most recent successful examples of classical biological control programmes. The high pressure by *T. sinensis* reflected on the native parasitoid community, and involved an expansion of its host-range with an occasional feeding also on non-target oak galls. The magnitude of the outcome of such introduction has been deeply investigated, and data about post-release evaluation of non-target effects are provided.

Keywords: Classical biological control, invasive exotic pests, Castanea spp., risk assessment

CO095

EXOTIC AND NATIVE NATURAL ENEMIES: HOW TO PLAN BIOLOGICAL CONTROL AND SAVE ENVIRONMENTS

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Biological control of invasive insect pests has a long history of success and it is considered as an ecologically sustainable and safe crop protection solution. Biological control has contributed significantly to reducing the environmental impacts of agricultural pest management, and in conserving indigenous species attacked by invasive insects. The global biological control market is estimated to grow at an annual rate of 13.6% during 2016-2021 forecast period. Its growth rate is attributed to high cost of agrochemicals and their negative impact on food quality and environment, the ban of insecticides as well as the increasing public demand for 'local' and organic foods. In addition, the increasing numbers of invasive insect pests resulting from globalization of trade and travel that reach new geographic areas without their regulating natural enemies present new opportunities for classical biological control to provide long-term cost-effective pest management. Implementation of biological control requires a comprehensive understanding of natural enemy ecology to predict the effectiveness of candidate biological control agents, and to ensure that negative effects on the environment are prevented. Continued concerns over potential non-target impacts, the consequences of the Nagoya protocol, and the non-harmonized regulation of biological control in Europe remain a challenge for classical biological control in Europe and thus, augmentative biological control using native natural enemies has been advocated even for field crops. The challenges and advantages of both approaches, classical and augmentative biological control, will be discussed using the example of the invasive pest *Halyomorpha halys* (brown marmorated stink bug) in Europe.

Keywords: Classical biological control, augmentative biological control, *Halyomorpha halys*

CO096

PARASITISM OF *PACHYCREPOIDEUS VINDEMMIAE* ON BURIED PEST PUPAE OF FRUIT ORCHARDS

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Introduction: Several Tephritoidea fly pests limit fruit production and after damaging flower buds or fruits larvae move into the soil to become pupa. The soil buried pupa stage can be controlled by the generalist ectoparasitoid wasp *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae). This parasitoid can regulate several Diptera species present in various agroecosystem but its efficiency is linked with host encounter probability and recognition distance. To better understand the mechanism underlying parasitism success we tested the impact of the soil composition, depth of the pupae, and the exposition time. We also compared the chemical profiles of both host and parasitoid pupae. Methods: Larvae of *Dasiops inedulis* (pest of passion fruit) and *Anastrepha striata* (pest of guajava) were reared to pupae. Groups of five pupae were set on peat or crop soil at different depths (from surface to 10 mm) and exposed during 24 and 48 h to five naïve parasitoids *P. vindemmiae* females on a Petri dish. Chemical analyses were done on several host species as *D. inedulis*, *A. striata* and also on the fly pest species *Musca domestica* and the Mediterranean fruit fly *Ceratitis capitata* used for parasitoid mass rearing and on the parasitoid *P. vindemmiae* pupae. For pupae chemical extraction we used dichlorometane and all sampled were injected in GC-FID. Results/Conclusions: *P. vindemmiae* parasitized *A. striata* and *D. inedulis* pupae. The rate of parasitism on both fly pupa species was the highest at the surface but not significantly different at 10 mm of depth therefore *P. vindemmiae* can find and parasitise buried pupae. Time of parasitoid exposition to pupae and type of soil did not influence the parasitism rate. The four host species did not share common compounds however the comparisons *A. striata vs* D. inedulus and M. domestica *vs* C. capitata shared common compounds. Parasitoid pupa and its unparasitised host shared several compounds. Results suggest that *P. vindemmiae* probably uses chemical information from other stages as larval trails to find the pupa and this information could be learnt during the preimaginal development. Parasitoid attraction experiments to larval trails and to detected pupa compounds should be conducted to confirm our results.

Keywords: Parasitoid, Diptera, orchards, pupa, chemical ecology

CO097

LONG TERM CHANGES IN THE COMMUNITIES OF NATIVE LADYBIRDS IN NORTHERN ITALY: IMPACT OF THE INVASIVE SPECIES *HARMONIA AXYRIDIS* (PALLAS)

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Since the mid-2000s, the exotic ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) has established itself in Northern Italy, raising serious concerns about possible impact on native ladybird species. In this study we compared the ladybird assemblages in 2015-16 with those sampled in 1995-96, before the arrival of *H. axyridis*. Samplings were carried out in the same sites and with the same methods for both periods. Aphidophagous ladybirds were monitored in hedgerows and herbaceous habitats at field margins by mechanical knockdown and sweeping net, respectively. The impact of *H. axyridis* on the structure of ladybird communities is different between arboreal and herbaceous habitats. *H. axyridis* is currently the dominant species in shrubs and trees, and all the native ladybird species taken together account for only approximately 1/3 of the total individuals sampled. On the other hand, the relative abundance of the exotic species in herbaceous habitats is low and a negligible impact on grassland ladybird communities has been detected. Among native species, *Adalia bipunctata* (L.), whose ecological niche largely overlaps with that of *H. axyridis*, suffered the most relevant decline between 1995-96 and 2015-16. The co-occurrence of *H. axyridis* invasion and the regression of *A. bipunctata* suggest a direct impact of the exotic species, because no other major modifications occurred in the studied areas between sampling periods.

Keywords: Coleoptera Coccinellidae, Biological control agents, Biodiversity, Agro-ecological schemes, *Adalia bipunctata*, Invasion biology

CO098

BIOLOGICAL CONTROL POSSIBILITIES OF AN INVASIVE SCALE INSECT IN ISRAEL: OPUNTIA COCHINEAL SCALE INSECT *DACTYLOPIUS OPUNTIAE*

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The Opuntia cochineal scale, *Dactylopius opuntiae* (Hemiptera: Coccomorpha; Dactylopiidae), an aggressive scale insect, was first reported in Israel from the Upper Galilee in 2013. The cochineal infests and kills the Indian-fig prickly pear, *Opuntia ficus-indica* (Cactaceae), a prominent plant in Israeli landscape. Several compounds of the neonicotinoid family were tested, and had proved effective as a preventive measure. The management attempt of the early outbreak was examined by application of inundated releases of the industrial mass reared lady beetle, *Cryptolaemous montrouzieri* (Coleoptera: Coccinellidae) was unsuccessful. Two natural enemies were collected in Mexico for the control of the scale in Israel; a predatory beetle, *Hyperaspis trifurcata* (Coccinellidae) and a predatory fly, *Leucopis bellula* (Diptera: Chamaemyiidae) was conducted. Both are known as specific enemies of member of the genus Dactylopius. Release *H. trifurcata* in the cochineal infested sites in the Galilee was successful with good recovery of the beetle in the release sites. Release of the predatory fly after the quarantine period is expected in the summer of 2018. Along the Mediterranean coast of the Western Galilee, local acclimatized *C. montrouzieri* populations begin to play a significant role in restraining the cochineal populations.

Keywords: Biological control, *Dactylopius opuntiae*, Opuntia ficus-indica, *Hyperaspis trifurcata*, *Leucopis bellula*

Maximising the Benefits of Biocontrol and Pollination Ecosystem Services

CO099

UNIVERSAL METHOD OF MOLECULAR SEXING OF LEPIDOPTERA

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Studies of ecology, evolution and physiology of insect as well as the development of pest management often assist with the necessity for sex identification. Morphological identification of sex in lepidopteran species is usually appropriate for adult stage but not juvenile stages. Lepidopteran insects have chromosomal sex determination (common ZW/ZZ). The main method of sexing for larval stage of Lepidoptera is karyotype analysis. The sex is identified according to presence/absence of sex heterochromatin. However, for eggs and young instars larvae this method requires high accuracy, which implies long time for insect preparation. Moreover, this method is inappropriate in the case of certain tissues or in frozen samples. For several Lepidopteran species, researchers have developed the molecular techniques for sex identification. These techniques are based on PCR detection of W chromosome in the samples. High genetic variability of W chromosomes makes it difficult to apply these techniques for different lepidopteran species. Currently there is no universal method of Lepidoptera sexing, which would be equally suitable for any type of samples, including frozen tissues. In order to identify sex of Lepidoptera species we propose a quasi-universal method based on the application of quantitative PCR.

This study was supported by Russian scientific foundation (grant # 17-76-10029).

CO100

INFLUENCE OF LANDSCAPE FEATURES AND MANAGEMENT PRACTICES IN OLIVE GROVES FOR OPTIMIZED PEST CONTROL

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The efficacy of natural enemies, such as spiders, to prey on pests within an agroecosystem can be affected by the management practices performed by farmers as well as local and landscape-scale features. Within-grove features such as local spatial structure and shelter availability could be crucial to maintain the spider populations within crops. Also, management practices and higher order landscape structures can affect the biodiversity of the community of spiders. The objectives of this work were to study the horizontal and vertical distribution of spiders along the grove, the effect of the size and number of stones occurring on the ground surface, the effect of the agricultural management, and landscape-scale features on the spider community, considering olive groves conducted under organic farming and integrated production systems in Trás-os-Montes (Portugal). Spiders were sampled using pitfall traps and sticky traps, and shaking tree branches. All the captured spiders were identified and grouped in guilds. We found an increasing pattern in species richness from the central area of the olive grove to the surrounding area. The distribution of functional groups followed a pattern both vertically and horizontally. The number of stones significantly influenced the biodiversity of spiders. A homogeneous surrounding landscape had a significant detrimental effect on the spiders diversity. The spatial distribution of the spider community within the olive crop as well as the surrounding landscape must be taken into account for pest management purposes oriented to enhance the population of spiders.

Keywords: Shrubland, predator, stones, canopy, trunk, ground.

CO101

POSHBEE - ADDRESSING BEE HEALTH AND SUSTAINABLE POLLINATION

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PoshBee is a consortium addressed at the recent EU call for Bee Health and Sustainable Pollination. Here I present the consortium, in particular focusing on its aims and objectives, and discuss opportunities for engagement and impact.

Keywords: Honey bee, bumble bee, solitary bee, pollination, agrochemicals, pathogens, parasites, nutrition

CO102

COMBINING ENTOMOPATHOGENIC FUNGAL ISOLATES AND BOTANICAL COMPOUNDS FOR CONTROL OF *FUNGUS GNAT* LARVAE (DIPTERA: SCIARIDAE)

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Fungus gnats (for example Bradysia impatiens) can be serious pests to plants grown in nurseries and greenhouses, and although various approaches for control are available, safer and more effective methods are desirable. Entomopathigenic fungi as biological control agents could provide an effective complementary system in an integrated pest management (IPM) strategy. The aim of this study was to determine the efficacy of entomopathogenic fungi and two botanical compounds on the development of *B. impatiens* larvae in growing medium. In a first series of bioassays, three selected fungal isolates *Metarhizium brunneum* (Cb15), *Beauveria bassiana* (EABb04/01-Tip) and one soil-borne endophytic fungus (*Acremonium strictum*) were applied at a rate of $1x10^7$spore/ml directly to the growing medium. After 24h, twenty fungus gnat larvae were introduced into each experimental cup, and adult emergence monitored about 2 weeks using yellow sticky cards. Larvae were more susceptible to infection by *M. brunneum* as compared to the other fungal strains; larvae exposed to *B. bassiana* successfully developed to adults. In a second series of bioassays, larvae were exposed to the substrate treated with two botanical microcapsules, containing mentha oil, and a biological insecticide (*Bacillus thuringiensis*, Bt). In this experiment, the mean total number of adults emerging from treatments ranged from 42% in Bt to 63% for the botanical compound, exhibiting an attractant effect for the larvae. Subsequent bioassays were set-up to determine the efficacy of a combination of *M. brunneum* and the botanical compound on larval development and adult emergence of fungus gnats in the growing medium. Data collected from this trial demonstrated that less adults hatched from these treatments compared to single compound treatments or the control. This result suggests that this botanical compound, although attractive to the larvae, may have an additive role on the efficacy of *M. brunneum*, resulting in a better efficacy to control fungus gnat larvae.

Keywords: *Bradysia impatiens*, Entomopathogenic fungi, fungal infection, adult emergence

CO103

USING NATURE TO INFORM THE DEVELOPMENT OF NOVEL TOOLS AND MOLECULES FOR SUSTAINABLE CROP PROTECTION

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A major challenge to both Science and Society is to increase agricultural productivity by an estimated 70% to feed an additional 2.3 billion people by 2050. Strategies to increase crop production must be both resilient and environmentally sustainable, requiring a paradigm shift in current practices. Developing strategies that are efficacious, but at the same time pose negligible risks to beneficial organisms, such as pollinators and those involved in biological control, is therefore both desirable and timely. Crops expressing genes encoding the entomocidal δ-endotoxin from *Bacillus thuringiensis* (Bt), first commercialised in the mid 1990s, have made a significant beneficial impact on global agriculture, and continue to do so. However, the potential for pest populations to evolve resistance, and lack of effective control of homopteran pests, requires alternative strategies to be developed. Over their co-evolution, insect antagonists such as predators and parasitoids have optimised a range of mechanisms and molecules to combat their hosts. To this end, neuroactive peptides/proteins linked to carrier molecules show significant promise, either for application as a biopesticide or when expressed in transgenic crops. Similarly, immunosuppressive strategies exhibited by parasitic wasps is currently being exploited to enhance the impact of BCA of insect pests. The potential of plant signaling molecules to enhance crop defence is yet another strategy being investigated. The use of RNA interference (RNAi) also provides an alternative approach to crop protection. This is a post-transcriptional process triggered by the introduction of double-stranded RNA (dsRNA), which leads to gene silencing in a sequence-specific manner. The presentation will provide an overview of the potential of these emerging technologies for effective control of crop insect pests. It will consider their biosafety and the different approaches to mitigate the potential for pest populations to evolve resistance, thus increasing their durability.

Keywords: Biopesticides, RNAi, Natural antagonists, Induced defence

CO104

ECOSTACKING: MAXIMISING THE BENEFITS OF ECOSYSTEM SERVICES

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Biodiversity at all levels from crop plant genetic variability to soil microbial assemblages, to botanical composition in a field, and to diversity of the agroecosystem landscape, have all been shown to affect the delivery of ecosystem services to crops. We should make full use of benefits accruable from ecosystem services via stacking and conservation of functional biodiversity in our cropping systems – by “ecostacking”. Stacking implies combining in an additive or synergistic manner the beneficial services of functional biodiversity from all levels and types. Ecosystem Service Providers (ESP) involved include primarily beneficial arthropods such as predators and parasitoids for the control of pests, seed feeders for weed control, and pollinating insects. Many other ESP also need to be involved, for example vertebrates, microbes, and the crop plants themselves (via induced resistance mechanisms). Microbes provide invaluable ecosystem services including pest, disease and weed control, either directly as components of suppressive soils, or as plant colonizers. Soils suppressive for plant pathogenic microbes have long been known, but awareness and potential of soils suppressive to insect pests have received only little attention. Endo- and epiphytes and their role in steering arthropod-plant interactions is under intensive study, while utilization of microbe-mediated bottom-up effects in crop protection is in its infancy. Increased knowledge about arthropod-plant interactions and factors modulating them is necessary in order to develop and support sustainable crop production. Basic research focuses on factors explaining the mechanisms involved in these interactions, identifying the key providers of biodiversity services, and quantifying the supply of ecosystem services such as biological control, pollination, and plant defense activators. A large amount of literature exists on individual components of functional biodiversity. The ideas, methods and techniques focusing on single components of the system have to be expanded and stacked, considering the pedo-climatic frame, the crop, the existing landscape elements, and the socio-economic conditions in the area, and combined in a meaningful and synergistic package approach. We therefore need to increase our knowledge on stacking of the most promising and productive ecosystem services into an operational, profitable, and sustainable whole.

Keywords: Functional biodiversity, biological control, pollination, crop protection, crop production

CO105

DOES OFF-CROP BIODIVERSITY HAVE AN IMPACT ON CROP YIELDS?

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Large investments have been made in Europe to support off-crop biodiversity in the hope that it will bring beneficial impacts in terms of better pollination and biocontrol services in the crop. The support mechanisms include field margin management and establishment of flowering strips around the fields. The impacts on crop yields, and/or on facilitating a reduction in pesticide use, have to my knowledge never been quantified. An in-depth understanding of the source-sink dynamics of ecosystem service providers and their movement between landscape elements is urgently needed to justify these support measures. In-depth knowledge is needed of the role of pollinators and insect predators, and how these are influenced by in-crop interventions and crop rotation. Addressing this knowledge-gap will enable the sustainable exploitation of ecosystem service providers in different agricultural systems, so reducing the need for chemical pesticides. In this presentation the current state of knowledge on the topic is reviewed.

Keywords: Flower strips, field margins, off-crop habitats, pollination, biocontrol, yield

CO106

DIRECT AND POLLINATOR-MEDIATED EFFECTS OF HERBIVORY ON PLANT FITNESS

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Herbivores can affect plant reproduction both directly and indirectly. Direct effects of herbivory result from consumption of plant tissues which may either directly destroy reproductive structures or cause resource limitation which, consequently, is decreasing the resources available for reproduction. Indirect effects of herbivory are due to corruption of the visual and olfactory signals that plants use to attract pollinators leading eventually to pollination limitation. Understanding the underlying mechanisms of how plant-herbivore-pollinator-interactions can affect plant fitness has important implications for agriculture, especially for insect-pollinated crops. Here, I present results from investigations studying the effects of herbivores and pollinators on the fitness of woodland strawberry (*Fragaria vesca*). Strawberry Leaf Beetles (SLB) feed both on strawberry leaves and flowers. In general, leaf damage is relatively well tolerated. However, after a certain threshold SLB-damage on leaves decreases both current and future sexual reproduction while clonal reproduction remains unaffected. Florivory by SLB leads to lower pollination success which, in turn, results in smaller fruits with more deformations. In addition to indirect effects, SLB-damaged flowers produce smaller fruits even when they are hand-pollinated, showing that florivory also has substantial direct effects on yield, independent of pollination. So far, our results demonstrate that SLB damage has not only direct negative effects on plant fitness but also that it interferes with pollination. Further studies are needed to test whether variation in plant resistance affects, not only the herbivore performance, but also pollinators.

Keywords: Crop wild relative, Diffuse interaction, ecosystem service, *Galerucella tenella*, *Galerucella sagittariae*, Florivory, Integrated Pest Management, integrated pest and pollinator management

CO107

SPECIFIC PLANT INTERACTIONS WITHIN AND BETWEEN SPECIES – UNDERESTIMATED MECHANISMS OF PEST CONTROL

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Sustainable insect pest management is often focused on using crop cultivars that are tolerant or resistant to pest attack. However, resistance breeding against aphids has not been sufficiently successful and, in addition, insect-pests can break this plant resistance. Botanical diversity, such as crop mixtures (intercropping) and variety mixtures, has been shown to improve ecosystem processes and function, including insect pest management. We have provided new evidences that co-existing plants interact with each other through different types of signalling, which may have implications for neighbouring plants morphology and physiology. The results of our investigations done under controlled condition in growing chambers and field, show that different ways of plant-plant interactions have effects on plant biomass allocation, their volatile emission, settling and population development of aphid and searching behaviour of aphid natural enemies. However, these bottom up tri-trophical effects were observed only in certain intercropping and variety mixtures, showing that plant-plant interactions depend on botanical composition. Results of our studies show that insects such as herbivores and their natural enemies are closely adapted to plant physiology and are highly sensitive to plant responses induced by different ways of plant-plant interactions. Our findings stimulate discussion on importance of understanding of complexity of plant-plant interactions which is underestimated inducing mechanisms of pest control and as potential of increasing effectiveness of ecosystem services in development of high productivity cropping systems.

Keywords: Aphids, plant-plant communication, induced plant responses, aphid settling, population development, botanical diversity,

CO108

FOOTHOLD MATTERS: BIOMECHANICAL INSIGHTS INTO PLANT-INSECT INTERACTIONS

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Insect-plant interactions at the surface level play a crucial role in the infestation of plants with phytophagous insects and therefore detailed knowledge about them can be used in biological control. Up to date, insect-plant interactions have been mainly investigated fusing the biochemical approach. Plant surface structures and insect attachment devices have been almost neglected in the context of crop pest management. However, insect attachment to plants has been previously considered as an important evolutionary feature for insect performance and fitness. Several experimental studies showed the strong influence of plant surfaces on the attachment ability of pest and beneficial insects. For example, plants covered with trichomes may repel beneficial insects (*e.g.*, ladybird beetles) or provide niches for specialised beneficial insects (*e.g.*, mirid bugs). On the basis of several case studies, an overview of plant-insect surface interactions from the perspective of insect attachment will be presented, suggesting new approaches for crop pest management, *e.g.*, (1) by modifying plant surface microstructures to make plants anti-adhesive for pest insects or (2) by supporting natural enemies using knowledge about their substrate requirements for optimal attachment and locomotion.

Keywords: Cuticle, insect attachment, pest management, plant protection, plant surface

Case Studies in Biological and Integrated Pest Control

CO109

BOX TREE MOTH (*CYDALIMA PERSPECTALIS*) AND ITS CONTROL IN THE UK

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The box tree moth caterpillar, *Cydalima perspectalis*, has been a serious problem on box (*Buxus spp.*) in mainland Europe since it arrived from Asia in 2006 and it is now threatening box in Britain. Box is an important plant in the UK: box topiary and parterres are key features of historic gardens, they are the dominant plant in some semi-natural habitats (*e.g.* Box Hill) and are very popular garden plants. At the moment box growers are already under pressure from box blight caused by the fungus *Cylindrocladium buxicola*. The caterpillar is capable of causing complete defoliation. Although slow growing, box should recover from a single defoliation event, however, significant defoliation over several years is likely to weaken plants. *C. perspectalis* larvae were first found in UK private gardens in 2011, since then the problem has spread and become established in London and surrounding counties. In the UK we have now documented and identified two native parasitoids capable of parasitising *C. perspectalis*; a Tachinid fly and a Chalcid wasp. Although unlikely to provide more than background levels of control they could still be of importance. Other control methods currently available to home gardeners are cultural, chemical and biological, all of which have potential drawbacks: being labour intensive and unfeasible, having negative impacts on non-target invertebrates (including pollinating insects) and possibly being incompatible with recommended control measures for the fungal pathogen, respectively. The RHS is surveying the spread of *C. perspectalis* and has ongoing research into the problems faced by box in the UK, with the eventual aim being to devise an integrated pest and disease management plan for this plant. Here we summarise our research to date.

Keywords: Native natural enemies, biocontrol, Buxus, box, box tree moth

CO110

HONEYDEW AS A FOOD SOURCE FOR BENEFICIAL INSECTS: A SWEET THAT CAN BECOME TOXIC

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Honeydew is a rich sugar excretion of hemipteran phloem-feeding insects. It is a ubiquitous carbohydrate source in most forests and agricultural lands. Over the last decades, it has been demonstrated that honeydew is the main carbohydrate source for beneficial arthropods, including pollinators, parasitoids, predators and ants in many agroecosystems when nectar is unavailable. As food source, honeydew can indirectly impact the population dynamics of herbivores through its nutritional value for parasitoids and predators. The strength of these effects will depend on the quality and availability of honeydew. The combination of these factors is expected to result in distinct scenarios that should be analyzed for each agroecosystem. This scenario, however, can change if honeydew-producers feed on plants treated with insecticides, survive to the treatment and excrete honeydew that contains insecticides or their metabolites. Herein, we have tested whether honeydew can be a new route of insecticide exposure to beneficial insects.

Keywords: Insecticides, plant phloem, beneficial insects, *Anagyrus pseudococci*, *Sphaerophoria rueppellii*

CO111

EFFECTIVENESS OF MATING DISRUPTION IN CONTROLLING INFESTATIONS OF THE VINE MEALYBUG, *PLANOCOCCUS FICUS*, IN VINEYARDS

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Mating disruption is nowadays widely used for a number of crop pests. This control strategy relies on reducing, if not suppressing, or delaying matings, thereby reducing the pest population growth potential. Mating disruption has been increasingly applied to control infestations of the vine mealybug, Planococcus ficus, even though the mode of action(s) remains unclear. Under laboratory conditions, the effects of increasing mating delays on reproductive and demographic parameters of P. ficus were investigated. The pre-reproductive increased proportionally with mating delay in females mated >7 after emergence, whereas fecundity was not influenced by female age at mating. The intrinsic rate of increase and the mean generation time also varied significantly on females mated >7 days. A three year field trial was subsequently carried out to study the effects of mating disruption applied in successive years on the pest population density. Mating disruption reduced the percentage of matings up to 66% and prolonged the pre-oviposition period up to 12 days. Our studies show that mating disruption negatively affects mealybug density by both delaying matings by >7 days and decreasing the number of matings. In addition, mating disruption increased its efficacy with consecutive applications, indicating that the repeated use of this pheromone-based control strategy would effectively concur to reduce insecticide spraying in vineyards.

Keywords: delayed mating, pheromone, fecundity, population density, life history

CO112

ENTOMOPHAGA MAIMAIGA IN EUROPE: TESTING THE SUSCEPTIBILITY OF GYPSY MOTH POPULATIONS OF ITALY

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Gypsy moth (*Lymantria dispar* L.) is probably the most important forest defoliator worldwide. The pest is native in Eurasia and North Africa, and in 1869 it was accidentally introduced in North America from France, becoming the most damaging defoliator of hardwood forests. In an attempt to contrast its diffusion in the USA, a highly virulent and host-specific fungal pathogen, *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) was introduced in 1910 from Japan, but it was recovered in the field for the first time only in 1989 after a collapse of L. dispar larvae in northeastern United States. The entomopathogen was then successfully introduced in 1999 in Bulgaria, from where it is now spreading throughout Eastern Europe, approaching also the Italian border. We analyzed the case of Italy, and Sardinia in particular, where the periodic gypsy moth defoliations of *Quercus suber* are cause of economically relevant loss in cork production. Therefore, after not finding evidence of the presence of *E. maimaiga*, we conducted a laboratory study to verify the efficacy of the fungus on Sardinian and Italian local populations of *L. dispar* in terms of toxicity (percentage morality), virulence (time to death), and fitness (reproduction success rate). Experiments were conducted employing a soil bioassay method involving temporary moth exposition to soil contaminated with *E. maimaiga* resting spores. Two different strains of *E. maimaiga* from Bulgaria and Croatia were tested. The fungus efficacy on other lepidopteran species, including *Malacosoma neustria*, were also evaluated to make a preliminary assessment of the environmental consequences following the possible introduction of this exotic microorganism. We discuss the results of bioassays highlighting significant differences in pathogenicity against different *L. dispar* Italian populations and between the two strains employed. We confirmed the expected low impact on non-target lepidopteran species.

Keywords: *Lymantria dispar*, entomopathogenic fungi, host specificity, bioinsecticide

CO113

CONTROL OF CHESTNUT MOTHS, *CYDIA FAGIGLANDANA* AND *C. SPLENDANA*, BY MATING DISRUPTION

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Chestnut moths, *Cydia fagiglandana* (Zeller) and *C. splendana* (Hübner) (Lepidoptera, Tortricidae), are main pest insects of chestnut fruits and their damages were dramatically increased by the spread of the oriental chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae), and to adverse climatic conditions. Chemical control of these carpophagous species is unsuitable due to the hazardous side effects that could occur in a complex ecosystem like a chestnut grove, particularly on beneficial insects. Among the alternative control strategies, sex pheromone-based control techniques are considered the most promising ones. During the last decade, and following the identification of the main components of the pheromone blends of these species, in a research program partially funded by the Plant Protection Service of the Campania Region, and with the technical support of Isagro for the development of the most efficient synthetic pheromone delivery system, the efficacy of 2 low-doses pheromone dispensers was compared. Both types of dispenser tested (Ecodian, Ecodian CT) were made of Mater-Bi, a completely biodegradable and compostable bioplastic. Ecodian is a hook-shaped dispenser, Ecodian CT is a wire made up of a cellulose core covered by Mater-Bi; 2,200 Ecodian dispensers, 600 or 900 meters of Ecodian CT (hanging 6 meters wire sections to the chestnut plants, at least 1.5 meters from the ground) per hectare were distributed, containing 45.0, 15.0 and 22.5 g of active ingredients, respectively. Field tests were conducted in 3 chestnut areas of the Campania Region (Montella, Roccadaspide, Roccamonfina); in each area, the 3 treatments (2 hectares per treatment) and an untreated control (2 hectares) were set up in chestnut groves of 8 hectares. Damage recorded in all pheromone-treated areas was significantly lower compared to the untreated control. The highest efficiency was provided by 900 meters of Ecodian CT and the damage was significantly reduced up to 83%.

Keywords: Lepidoptera, Tortricidae, Chestnut fruit, sex pheromones, biodegradable dispensers

CO114

HOST FEEDING AS A TOOL TO IMPROVE BIOLOGICAL CONTROL OF THE ASIAN CITRUS PSYLLID BY TAMARIXIA RADIATA?

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The parasitoid *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) is being used world-wide for the biological control the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae). The parasitoid is strongly synovigenic, as it is born with very few mature eggs. Synovigenic insects need to feed on host haemolymph to mature additional eggs, and are able to resorb mature eggs to allocate resources toward maintenance. We investigated the effect of host feeding on parasitism behavior, longevity and egg load dynamics, and estimated egg maturation and resorption rates. We showed that, whilst host feeding does not increase survival or longevity, it results in increased parasitization rates when parasitoids are seven days old (the age at which they are usually released in California), that a single host meal leads to an average gain of three eggs and accelerates the egg maturation rate. We argue that the host feeding gains could be exploited at the mass rearing level to improve the nutritional status of mass reared *T. radiata* females upon release. We modeled parameters gathered by laboratory experiments to predict the effect of pre-release host feeding on the foraging and parasitization behavior of *T. radiata* in the field, and performed field cage experiments to test the model. We will discuss the effect of host feeding on the efficacy of augmentative biological control of ACP from a theoretical and practical perspective.

Keywords: Citrus, *Diaphorina citri*, mass rearing, synovigeny

CO115

ALEUROCANTHUS SPINIFERUS, AN ALIEN INVASIVE THREAT TO EUROPE. ASSOCIATED BACTERIAL COMMUNITY AND NATURAL ENEMIES

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Aleurocanthus spiniferus also known as orange spiny whitefly (OSW), is a pest native to tropical Asia that in the last century has spread throughout Asia, reaching Africa, Australia, and Pacific islands. In 2008 the first European OSW population was recorded in Apulia region (South East Italy) and allowed EPPO to add the species as a quarantine threat to Europe now in the A2 list. In the following years OSW spread and invaded new territories of Italy, Croatia and Montenegro. Although OSW polyphagy is already well-known, new associations with autochthonous and allochthonous plants have been reported showing its host-shifting ability. To counteract an upcoming pan-Mediterranean invasion updated bio-ethological information of the pest and the role of possible natural enemies are essential to implement a correct IPM strategy. Field samplings have been aimed at the identification of natural enemies and the evaluation of their efficacy. Furthermore, through insect small-RNA sequencing and by Denaturing Gradient Gel Electrophoresis (DGGE) technique coupled with 16S-rRNA gene sequencing, the primary symbiotic bacteria of OSW have been identified. Sampling on natural enemies highlighted the presence of predatory species belonging to the Coccinellidae family. Besides to the almost ineffective populations of *Oenopia conglobata* and *Clithostetus arcuatus*, new findings detected scattered *Delphastus sp.* populations along the western coast of Italy. Both adult and larvae of this ladybird species preyed OSW developmental stages. The evaluation of the role of *Delphastus sp.* as biocontrol agent is underway. The first study on OSW microbiota allowed to find symbiotic bacteria commonly associated with the genus Aleurocanthus: *Portiera sp.*, *Serratia sp.*, *Wolbachia sp.*, *Rickettsia sp.* and, although sporadically, other species. Further studies will target the functional role of these symbionts to develop an effective IPM tailored for Countries at risk.

Keywords: *Ailanthus altissima*, Aleyrodidae, Citrus, Endosymbiotic, endocytobiotic bacteria Hemiptera

CO116

INTEGRATED PEST MANAGEMENT OF THE NEW INVASIVE CITRUS PEST *DELOTTOCOCCUS ABERIAE* (HEMIPTERA: PSEUDOCOCCIDAE)

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Delottococcus aberiae (De Lotto) (Hemiptera: Pseudococcidae) is a new pest for citrus that has recently invaded the Mediterranean region. This mealybug causes severe distortion and size reduction on developing citrus fruit and leads to severe economic loses. Herein, we describe different strategies to control this invasive pest without disturbing the current Integrated Pest Management program in citrus. We first studied the native natural enemies to develop a biological control strategy. Among the native natural enemies, not any hymenopteran parasitoid species was able to successfully parasitize on *D. aberiae* because the eggs laid were encapsulated by the host. The predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) was commonly observed feeding on *D. aberiae* colonies but its population increased only after *D. aberiae* had already damaged the fruit. Augmentative releases of *C. montrouzieri* larvae in March successfully reduced *D. aberiae* populations before the damage occurred. We then, developed a sampling protocol and calculated the economic thresholds in order to reduce the number of insecticide treatments. We recommend sampling 275 fruit bimonthly using a binomial sampling method between petal fall (April) and July. Environmental Economic Injury Levels and Economic Injury Levels were calculated as 7.1% and 12.1% of occupied fruit in spring, respectively.

Keywords: *Cryptolaemus montrouzieri*, biological control, hymenoptera, encapsulation, economic thresholds

CO117

SUPPRESSION OF HIGH AND LOW DENSITY POPULATIONS OF MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE) THROUGH MASS TRAPPING WITH TRIMEDLURE (TML) DISPENSERS IN COFFEE IN HAWAII

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To assess the potential to suppress Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), via mass trapping with Trimedlure [TML], we compared fly catch (as catch per trap per time period) provided by either a novel, solid, triple-lure dispenser with TML, methyl eugenol, and raspberry ketone [TMR] or solid TML plugs, both without insecticides, in addition to Biolure bait stations. Work was done in a coffee plantation that had a dense *C. capitata* population. Three treatments were compared: (1) TMR or TML (50 traps per ha), (2) Biolure (50 traps per ha), (3) TML (25 per ha) or TMR (25 per ha) + Biolure (25 per ha), and (4) an untreated control. During coffee season, based on *C. capitata* captures (mean flies per trap per wk) inside plastic McPhail traps, all treatments were significantly different than the control: Biolure (9.57)=TMR (11.28)=Biolure +TMR (13.50) <Control (36.06 flies/trap/wk). During noncoffee season, all treatments were significantly different than the control and TML was significantly lower than Biolure (wax matrix bait stations): TML (0.95) <Biolure (1.43)=Biolure +TML (1.77) <Control (2.81 flies/trap/wk). Surprisingly, captures were not lower in plots treated with combinations of Biolure + TMR or TML, compared to individual plots with Biolure or TML or TMR alone. Mass trapping with either TML or TMR dispensers deserves further study as a component of Integrated Pest Management programs for *C. capitata* in Hawaii and may have global potential for management of *C. capitata*.

Keywords: Fruit flies, *Ceratitis capitata*, male lures, male annihilation, Integrated Pest Management

Session 4.
Chemical Ecology and Multitrophic Interactions

Invasive Alien Species, Population Genetics, Infochemical Network

CO118

CHARACTERISATION OF VOLATILE COMPOUNDS RELEASED BY APPLE TREES INFESTED BY DIFFERENT APHID SPECIES

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Herbivore-induced volatile organic compounds (VOCs) are released by plants after insect attack and are known to play important ecological roles, regulating several ecosystem interactions, such as plant-pest, plant-plant, and plant-predatory interactions. In this research, we chemically characterise the 'bioactive' natural compounds released by apple trees, cv. Gala and Cv. Fuji, infested by three aphid species with the aim to understand possible coevolution at species level and to enhance the attraction of natural pest enemies in apple orchards. Apple foliage infested with aphid colonies of rosy apple aphid, *Dysaphis plantaginea*, woolly apple aphid *Eriosoma lanigerum* and apple aphid *Aphis pomi*, were sampled in organic-managed apple orchards of Laimburg Reserch Station, Italy. The headspace of enclosed branches was collected by the closed-loop-stripping-analysis method and each compound was identified with a gas chromatographer coupled with a mass spectrometer. Preliminary results showed that each aphid species induced the release of specific pattern of volatiles, an indication of a species- specific responses of apple trees to each aphid species. P-Cymenes were released only by Cv. Fuji infested with *E. lanigerum*, while benzaldehyde and beta-farnesene were released only by Cv. Gala infested with *Aphis pomi*. Caryoplyllene was realised by all invested apple trees but was significantly higher only in Cv. Gala infested with *D. plantaginea*.

Keywords: *Dysaphis plantaginea*, Eriosoma lanigerum, Aphis pomi, chemical ecology.

CO119

TOMATO LEAVES UNDER BIOTIC STRESS: CAN WE DETECT SPECIFIC VOC BLENDS IN THE GREENHOUSE?

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Plants emit specific blends of organic volatile compounds when attacked by herbivores (herbivory-induced plant volatiles; HIPV) or pathogenous fungi (pathogen-induced plant volatiles, PIPV). The majority of previous studies has been conducted under laboratory conditions. Hence, we conducted two greenhouse experiments to investigate specificity and resilience of volatile blends in a more challenging environment. In a comprehensive approach, using solid-phase microextraction (SPME) to collect samples, we detected a total of 89 compounds and gathered very detailed information on constitutively emitted volatiles, HIPV associated with two sap-feeding insect herbivores, *Macrosiphum euphorbiae* and *Bemisia tabaci*, and PIPV associated with the biotrophic fungus *Oidium neolycopersici*. Using orthogonal projection on latent structures discriminant analysis (oPLS-DA), we could distinguish the various blends. This was partly due to absence and presence of certain biomarker compounds, partly due to changed ratios. Our greenhouse experiments confirmed results from laboratory studies regarding species-specificity: distinct *B. tabaci*-HIPV were emitted from infested plants and their composition changed over time, correlating with the presence of different developmental stages of the herbivore. *O. neolycopersici*-infection lead to the emission of PIPV distinct from the sap feeder HIPV and from constitutively emitted blends, although they share the majority of compounds. Between the two experiments, HIPV were more similar than constitutive blends, indicating a high resilience of HIPV and high phenotypic plasticity regarding constitutive blends. The latter may be more sensitive to biotic and abiotic changes. Ecologically, a variable constitutive blend may impede detection by herbivores while resilient HIPV blends should support indirect defense by natural enemies.

Keywords: Plant VOC, *Bemisia tabaci*, *Macrosiphum euphorbiae*, *Oidium neolycopersici*, *Solanum lycopersicum*

CO120

TRANSLATING OLFACTOMES INTO ATTRACTANTS: SHARED VOLATILES PROVIDE ATTRACTIVE BRIDGES FOR POLYPHAGY IN FRUIT FLIES

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Tephritidae is a widespread and speciose fruit fly taxon, of which many members are invasive fruit pests. Most of these species display defined, but overlapping ecological niches, of which we here analyzed the olfactory correlate. Using the volatilome (headspace) of four different host fruits and antennal recordings, we compare the fruit-odor olfactome of closely and distantly related tephritids with overlapping and distinct ecologies. Of a total of 349 different volatiles, 111 elicited antennal responses, 64 of which in all four fly species. Olfactomes of the four species were distinct, with a significant divergence bias correlated with ecological niche. Irrespective of relatedness or ecology though, the presence of a volatile across the four fruits ('sharedness') strongly increased the probability of detection in all species. This held even true for the 'unrelated' *Drosophila melanogaster* (DoOR repository-based analyses): the likelihood of detection by *D. melanogaster* increased with sharedness of a volatile across fruits, as well as its sharedness of detection by tephritid species. Next, to translate olfactomes into ecologically relevant behavior, we conjectured that shared volatiles signify 'host' to the 'nose' of the fruit fly and induce attraction. Therefore, we tested the various fruit fly species to blends of 6 (detected by all tephritid species, present in all fruits) and 11 (all tephritid species, present in ≥3 fruits) compounds in a multi-choice olfactometer. Indeed, nanogram quantities of these blends in mango ratios were very attractive for tephritids and even for *D. melanogaster*, exceeding the attractiveness of fruits. Quantitative whole antennal recordings en lieu of, or complementary to, bottom-up molecular neurogenetic approaches, enables comparative olfactomics in non-model species, and facilitate interpretation into evolutionary, ecological and applied contexts.

Keywords: Fruit fly, Tephritidae, attractants, olfactome, niche, olfactory evolution

CO121

SCANNING ELECTRON MICROSCOPY OF ANTENNAL SENSILLA OF *AROMIA BUNGII* (COLEOPTERA, CERAMBYCIDAE) ADULTS AND ELECTROANTENNOGRAM RESPONSES TO VOLATILE ORGANIC COMPOUNDS

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The red-necked longhorn beetle, *Aromia bungii* (Faldermann) (Coleoptera, Cerambycidae) is a major pest of *Prunus species* that has recently been recorded in several countries in Europe. To contribute to the knowledge of the olfactory system of this pest, the external morphology and distribution of antennal sensilla of both sexes were studied by scanning electron microscopy (SEM). Moreover, the chemoreceptivity of the apical (9th-7th), middle (6th-4th), and basal (3th-1nd) groups of flagellomeres to 1 mg of stimulus loads of 11 volatile organic compounds (VOCs), previously reported as bioactive towards other xylophagous beetles, was determined using electroantennography (EAG). In both sexes the antennae are filiform consisting of a basal scape, a pedicel and a long flagellum composed of nine flagellomeres. Based on their morphology five types of sensilla chaetica, three types of *Sensilla basiconica*, one type of sensilla trichodea and Böhm bristles were identified on the antennae. The strongest antennal stimulants were sulcatol, nonanal, sulcatone, and 2-phenyl-ethanol in males and sulcatone, sulcatol, 3-pentanol, and nonanal in females whereas 1-ethanol and glycerol were the weakest antennal stimulants in both sexes. The mean EAG responses of the antennal apical part were significantly higher ($P<0.05$; Tukey test) than those of the middle and basal parts for 10 compounds in females and 8 in males. *Sensilla basiconica*, inferred to have an olfactory function, are present in all flagellomeres but with a patchy distribution and a variable concentration. Moreover, the female 9th flagellomere presents areas where the *Sensilla basiconica* are organized in very gathered clusters. These are assumed to function as an enlarged odor-sensing area advantaging long-distance olfactory detection, hence supporting the significantly ($P<0.05$; t-test) higher female EAG responses to 3-pentanol, 1,8-cineol, and ethyl acetate.

Keywords: Red-necked longhorn beetle, SEM, EAG, VOCs

CO122

ARE UNCOMMON DITERPENES MEDIATING THE HOST LOCATION PROCESSES OF THE STINK BUG *BAGRADA HILARIS* TOWARD *BRASSICA OLERACEA* VAR BOTRYTIS SEEDLINGS?

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The painted bug, *Bagrada hilaris* Burmeister is a stink bug native to the Asia and Africa and invasive in USA, Mexico, and more recently, South America. This pest causes major damage on various vegetable crops belonging to the Brassicaceae family. Young seedlings of *Brassica spp.* at the cotyledon stage are particularly susceptible and vulnerable to *B. hilaris* feeding activity. The olfactory cues exploited by this insect for location of its hosts have not yet been identified. In this study, we investigated the role of volatile organic compounds (VOCs) in the attraction of *B. hilaris* individuals toward one of its main host plants, *Brassica oleracea* var botrytis at the seedling stage. Crude host plant VOCs and liquid chromatography fractions thereof were bioassayed to locate the active compound(s). Thus, volatiles from *B. oleracea* 7-day old seedlings were collected and bioassayed with *B. hilaris* adults and late stage nymphs, using electroantennographic techniques (EAG) and vertical Y-shaped olfactometers. The results of EAG and behavioural experiments indicated that *B. hilaris* adults and nymphs responded to the crude volatiles of *B. oleracea* seedlings, and to a nonpolar fraction of extract, whereas there were no responses to the more polar fractions. GC-MS analysis of the nonpolar fraction showed the presence of several as yet unidentified diterpene hydrocarbons. These chemicals appear to act as the key mediator in this insect-plant interaction, and could be good candidates for use in lures for *B. hilaris* monitoring in the field.

Keywords: Painted bug, host plant, VOCs, EAG, olfactometer

CO123

VOC: POWERFUL "PLANT-TO-PLANT" ELICITORS OF DEFENCE

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In the last few decades, incredible advances have been made in characterising the nature, the origin and the function of plant volatile compounds. These molecules/blends regulate important functions of plant life linked to primary and secondary metabolism. Of particular interest is the role of these compounds in plant defence against insects (direct and indirect), as determined by constitutive or induced release, and in plant-to-plant communication. Volatile communication inducing a defensive response has been assessed for conspecifics, congeneric and interspecific plant species and appears to be a quite common phenomenon. The characterization of compounds/mixtures involved in these multitrophic interactions is of great interest for its possible exploitation in IPM protocols aiming to enhancing biological control. From an ecological perspective, priming induced in neighbouring receiver plants could result in an evolutionary driving force towards plants more able to eavesdrop on signals and then more ready to be primed against invaders.

Keywords: Direct defense, indirect defence, IPM, biological control

CO124

EXPLOITING CONSERVATION BIOLOGICAL CONTROL BY MANIPULATING THE CHEMICAL ECOLOGY OF MULTITROPHIC INTERACTIONS USING GM

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From an understanding of the signalling processes of tritrophic interactions we can exploit natural populations of carnivorous insects, such as hymenopterous parasitoids, for sustainable pest management. The prerequisites for such sustainability, are that natural parasitoid populations are maintained and that foraging by the parasitoids is enhanced before pest threshold populations are reached. Success in this approach is evidenced in the push-pull system developed for sub-Saharan cereal crop protection against lepidopterous stemborers. Natural parasitoid populations are maintained by botanically diverse agro-ecosystems incorporating wild host plants of the herbivores. The parasitoids are then lured into cereal crops by intercrops releasing foraging semiochemicals, such as C11 and C16 homoterpenes (more correctly termed tetranorterpenes), naturally induced by feeding on the cereal hosts. For industrial agriculture, induction or priming mechanisms are targeted and these processes will need to be managed by use of plant defence elicitors applied to the crop or released by sentinel plants. Progress towards accomplishing these objectives by GM will be described. The potential depletion of natural parasitoid populations can be accommodated by increased ecosystem services in the agro-ecological regions involved and will be costed as part of a "land sparing" rather than "land sharing" policy. In addition to the use of foraging cues from crop plants, signals for dispersing parasitoids from production sites created by land sparing are required and may be obtained from signalling involving systems at the fourth trophic level and also manipulated by GM.

Keywords: Conservation, biological control, chemical ecology, multitrophic interactions, genetic modification, ecosystem services

CO125

SEMIOCHEMICALS TO CONTROL THE WALNUT HUSK FLY *RHAGOLETIS COMPLETA*

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Most European walnut producers have to deal with the recent introduction of the Walnut Husk Fly, *Rhagoletis completa* (Diptera, Tephritidae), that causes severe economic losses, especially in biological productions. In orchards where *R. completa* is present and uncontrolled, losses in walnut yields can reach up to 80%. As a consequence, there is a need for developing environment-friendly methods of control. In this research, we evaluated the efficiency of two different semiochemical blends to trap *R. completa* in walnut orchards. The first semiochemical blend consisted in walnut fruit volatiles, previously collected and identified from two walnut varieties. The second semiochemical blend was made of the putative male sex pheromone, made of two lactones, and previously collected from *R. completa* males. In field both semiochemical blends were associated with yellow sticky traps. The assays were conducted in France, in a total of 53 orchards, in 2015, 2016 and 2017. The first blend (made of fruit kairomones) was highly attractive for both male and female *R. completa* in a laboratory assay, but did not enhance the number of fly captures in the field. On the other hand, sticky traps associated with the putative sex pheromone captured up to 10 times more fruit flies each week during the entire season. The total number of captured flies was also more important than with a mass trapping system baited with food attractant. These field results are promising for *R. completa* monitoring and mass trapping.

Keywords: Fruit flies, Volatile organic compounds, Sex Pheromone, Integrated pest management, Walnuts

CO126

EGG–LAYING BEHAVIOR OF *DROSOPHILA SUZUKII* WITH EVIDENCE OF AN OVIPOSITION MARKING PHEROMONE

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Drosophila suzukii Matsumura (Diptera: Drosophilidae) is an invasive fly native to Eastern Asia that has successfully invaded Europe and the Americas since its first detection in 2008 outside its native range. Its rapid spread and establishment is largely attributed to its ability to exploit for oviposition multiple intact host fruits ripening on plants; this ability is due to the presence of a sharp, enlarged ovipositor. The mechanisms of the oviposition site choice of *D. suzukii* are a matter of hypothesis. The aim of this work was to elucidate behavioral and chemical aspects of short-range site selection of this species. Video recording evidenced several discrete and repeated steps during oviposition. In particular, the final step is the release of an anal secretion over the fruit surface near the oviposition site. Choice and non-choice tests indicate oviposition preference towards egg-infested fruits, suggesting a role of the released liquid as a marking pheromone attracting multiple ovipositions. The knowledge gained with this study may accelerate establishment of control strategies based on the interference and disruption of the *D. suzukii* communication during the oviposition processes.

Keywords: Spotted wing drosophila, oviposition behavior, pheromone, IPM

Plant-Microbe-Insect Interactions (COST Action FA1405 Crop-Arthopod-Microorganism (CAMo) Interactions)

CO127

ARE APHID VECTORS MANIPULATED BY PLANT VIRUS THROUGH CHEMICAL CUES?

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Vector-borne plant viruses can manipulate their host-plant for their own benefit by attracting the vectors and facilitating their acquisition through changes in chemical and visual cues of the infected host plant (indirect effects on vectors). Moreover, increasing evidence suggests that vector-

borne viruses can modify the vector behaviour directly when the vector is carrying the virus (direct effect on vectors). Our main question was to know whether the indirect and direct effects of virus on vectors systematically promotes virus propagation". In other words, do "indirect and direct effects" mean "indirect and direct manipulations"? The purpose of this study was to investigate potential combined indirect and direct effects of a plant virus on the behaviour of its vector in the pathosystem *Arabidopsis thaliana* / Turnip yellows virus (TuYV) / *Myzus persicae*. TuYV is transmitted by aphids in a circulative mode and acquired by the insects when ingesting sap from an infected plant. Non-viruliferous aphids exhibited an altered feeding behaviour on virus-infected plants (indirect effect) but in a way not conducive to virus dispersion (reduced sap ingestion). Accordingly, chemical analysis comparing infected and non-infected plants showed that secondary metabolites in leaves were qualitatively significantly different. Viruliferous aphids (compared with non-viruliferous aphids) exhibited an alteration of their feeding behaviour on healthy plants, showing a direct effect of the plant virus also in a way contrary to a promotion of its dispersal. Finally, despite direct and indirect effect were observed, the only element in favour of aphid manipulation by the virus was that viruliferous aphids exhibited increased intrinsic velocity. To summarize, our results highlighted both indirect and direct effects of the virus on its host-plant and its vector respectively, but these two alterations may not be considered as virus manipulation of the aphid host because virus dispersion was not promoted.

Keywords: aphid, behaviour, metabolites, plant virus, arabidopsis, Electropenetrography

CO128

TURNIP YELLOWS VIRUS INFECTION INDUCES CHANGES IN EMISSION OF VOLATILE COMPOUNDS AND CONTRASTED APHID VECTOR BEHAVIOR IN TWO HOST PLANTS

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Vector-borne plant viruses can alter plant phenotype and vector behaviour in such a way that virus transmission may be affected. We investigated the deregulations induced by a circulative aphid-transmitted virus in two host plants and their potential impact on its transmission by the aphid vector. The pathosystem is composed of Turnip yellows virus (TuYV, genus *Polerovirus*, family Luteoviridae) which is efficiently transmitted by the polyphagous aphid *Myzus persicae*, and two Brassicaceae host plants, the cultivated *Camelina sativa* and the model plant *Arabidopsis thaliana*. Infected or non-infected plants were used for volatile analyses combined with choice tests, in order to investigate the potential impact of virus infection on vector preference. We observed that aphid behaviour varied depending on the plant species. Whereas non-viruliferous aphids showed no preference for infected or non-infected *A. thaliana*, aphids were more attracted to TuYV-infected *C. sativa*. These results correlated with a tendency of infected *C. sativa* to release a different blend of volatiles. Analysis of volatiles emitted by infected *C. sativa* identified two compounds which over-accumulated and 10 less abundant when compared to non-infected plants. Choice tests involving *C. sativa* infected with TuYV mutants suggested that several viral proteins are required to induce metabolic deregulations in infected *C. sativa*, which may be responsible for the aphid attraction. These exploratory data suggest that the same virus is able to trigger different metabolic changes in two plant species within the Brassicaceae family. These modifications induced contrasting changes in the aphid behaviour, which outcome may have an important impact on virus transmission by aphids and on disease outbreak.

CO129

THE ASSOCIATION BETWEEN DROSOPHILA SUZUKII AND HANSENIASPORA UVARUM

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Drosophila suzukii is a highly invasive pest of soft fruit and berries in Europe and North America. Unlike *D. melanogaster*, which oviposits on overripe fermenting fruit, *D. suzukii* exploits a different environmental niche. Studies on Drosophila-yeast association show that yeasts affect drosophila physiology, behavior and immunity. The yeast, *Hanseniaspora uvarum* is considered to be widely associated with *D. suzukii*. We investigated the impact of *H. uvarum* on *D. suzukii* and vice versa. Further, we also report the nature of association between *H. uvarum* and *D. suzukii*. The larval and adult fly attraction and oviposition to *H. uvarum* is significantly higher. *H. uvarum* as a food resource is sufficient for the fly development. The antagonistic effect of H.uvarum to suppress the gray mold *Botrytis cinerea* benefits the fly. A significant negative effect of the *Botrytis cinerea* on the larval attraction, fly development and ovipostion are observed. The yeast suppresses mold growth and benefits in fly development whereas the fly presence benefits in yeast growth. The mold and yeast growth progression in presence/absence of fly, quantified by qPCR, confirms an association between yeast and fly. We measured the ethanol production by H uvarum and the development and survival of flies on different concentration of ethanol. The significantly low amount of ethanol production by *H. uvarum* and low alcohol tolerance of *D. suzukii* explains their association and habitat selection. *D. suzukii* also benefits the yeast by vectoring it from one place to the other. From this study we conclude that *H. uvarum* and *D. suzukii* benefits from each other and have a mutualistic-association between them.

Keywords: *Drosophila suzukii*, invasive pest, *Hanseniaspora uvarum*, *Botrytis cinerea*, ovipositon, antagonistic effect, qPCR, mutualistic-association

CO130

IMPACT OF THIRD TROPHIC-LEVEL SYMBIONTS ON PLANT-MEDIATED INDIRECT INTERACTIONS

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The importance of mutualistic symbioses is increasingly recognized in plant responses to herbivory as well as in plant-mediated interactions. It is well established that beneficial microbes such as mycorrhizal fungi and plant growth-promoting bacteria can play a role in plant responses to herbivory. Symbionts associated with herbivorous insects are also known to affect the way the herbivore interacts with its food plant by manipulating plant-physiology for the benefits of their herbivore hosts. However the role of insect-associated symbionts has received less attention in a multi-trophic perspective. Here, we will address the effects of symbionts associated with insects at different positions in the community structure. Using as model system a cabbage-based food-web system, we show that insect-associated symbionts induce phenotypic changes in the plant, with consequences for other insect community members. We argue that insect-associated symbionts can play an important role in plant-mediated interactions highlighting a fascinating complexity of terrestrial-ecological networks.

Keywords: Symbioses, plant-mediated interactions

CO131

TRICHODERMA STRAINS IN THE BIOCONTROL OF INSECT PESTS

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Fungal root symbionts can induce different levels of enhanced protection against fungal plant pathogens. However, the induction of plant systemic resistance appears to confer some degree of protection also against insects, which has been poorly investigated. Here we report how selected strains of the biocontrol agent Trichoderma may be useful in the control of tomato pests with different attack strategies and sensitivities to defence response: the noctuid moth *Spodoptera littoralis* Boisduval and the aphid *Macrosiphum euphorbiae* (Thomas). We observed different outcomes of the tomato plant – Trichoderma interaction against S. littoralis, including negative impact on larval development and pupation rate, depending on the strain used. The interaction between tomato plant, root symbiont and *M. euphorbiae* resulted, depending on the fungal strain used, in lower aphid fertility and longevity. Accordingly, the expression of defence-related genes is multifaceted and dependent on the applied fungal strain. While the impact on aphids can be related to the up-regulation of genes involved in oxidative burst and in early defence reactions, the negative performance of *Spodoptera* larvae can be related to late defence genes. The attraction of natural enemies, namely the aphid parasitoid *Aphidius ervi* Haliday, may as well be enhanced by selected Trichoderma strains, both in presence and in absence of aphids. The parasitoid flight is associated with the release of VOCs and the up-regulation of genes involved in the salicylic acid pathway and in terpenoid biosynthesis. Our findings suggest that there are different factors concurring in the final outcome of the plant/symbiont association. It seems that the dose of inoculum of the symbiont and the temperature are important aspects to be further investigated, in order to reduce the current unpredictability of the control effects against insects of Trichoderma strains.

Keywords: Crop-Arthropod-Microorganism (CAMo) interactions, PGPF, plant induced defence, *Trichoderma harzianum*, *Trichoderma atroviride*

CO132

INTERACTION OF BEETLE POLYGALACTURONASES WITH PLANT INHIBITORY PROTEINS

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The herbivorous mustard leaf beetle *Phaedon cochleariae* feeds on brassicaceous plants and possesses various digestive carbohydrases targeting plant cell wall polysaccharides. Amongst those, polygalacturonases (PGs) hydrolyse the cell wall polysaccharide pectin. Plant-derived, cell wall-associated polygalacturonase-inhibiting proteins (PGIPs) counteract microbial PGs and thus contribute to the plant´s defence against phytopathogens. However, direct interactions between beetle PGs and plant inhibitory proteins have not yet been investigated. We performed interaction studies of *P. cochleariae* PG family members with crude cell wall protein extracts of the beetle's food plant Chinese cabbage (*Brassica rapa ssp.* pekinensis). Putative PGIPs and other leucine-rich repeat (LRR) proteins were identified by MS/MS representing candidates for beetle PG inhibition. Heterologous expression of candidate proteins allows for the characterisation of their specificity and inhibitory activity towards beetle PGs *in vitro*. Both PGs and PGIPs belong to multigene families that are believed to have been shaped by an evolutionary arms race. The number of PGIPs in brassicaceous plants ranges from two in *Arabidopsis thaliana* to nine and 16 in Chinese cabbage and rapeseed (*B. napus*), respectively. The oligophagous *P. cochleariae* can be reared on *A. thaliana* and, in contrast to Chinese cabbage, both AtPGIPs have been extensively characterised. The variety of knockout mutants and versatile molecular tool box available motivated us to include *A. thaliana* to investigate the potential role of PG – PGIP interactions *in vivo*. We used mutants lacking either AtPGIP1 or AtPGIP2 to analyse (i) beetle life history and beetle PG regulation in the presence or absence of putative inhibitors and (ii) the regulation of plant PGIPs in response to beetle feeding. Integrating data from both the model *A. thaliana* (easy to manipulate) and the beetle's food plant Chinese cabbage (ecologically more relevant) will shed light on the role of plant PGIPs in defence against herbivorous beetles in general.

Keywords: Plant cell wall-degrading enzyme, PCWDE, polygalacturonase, PG, polygalacturonase-inhibiting protein, PGIP, plant defence, herbivory, beetle, Coleoptera

CO133

MICROBES ASSOCIATED WITH INSECT EGGS: DO THEY AFFECT PLANT ANTI-HERBIVORE DEFENSE?

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Several microbial symbionts of oviparous insects may be transgenerationally transferred directly via the egg stage or by being released by the parental generation at sites where the offspring will feed. Eggs deposited by herbivorous insects onto the leaves of their host species can elicit defensive plant responses targeting the eggs and/or the hatching larvae. Elicitors of these plant responses are present in secretions which the insect female releases with the eggs. However, while plants are well known to respond to microbes, it has not been investigated so far whether bacteria associated with insect eggs contribute to eliciting plant defence against hatching larvae. To address this question, we treated *Pieris brassicae* with antibiotics and analysed whether the treatment affected the microbial load of the eggs. Furthermore, we compared the effect of control eggs and eggs laid by antibiotics treated parents on plant defence against larvae. We used *Brassica nigra* and *Arabidopsis thaliana* as host plants. Our study revealed that eggs of *P. brassicae* carry only a low and very variable bacterial load. Nevertheless, the antibiotics treatment reduced the microbial load by about fourfold. As shown previously by us, larvae feeding on plants which have received eggs from untreated butterflies gain less larval und pupal weight than those feeding on egg-free plants, indicating that egg-deposited plants defend more efficiently against larvae than egg-free plants. However, this increase in defence efficiency is significantly attenuated when antibiotics treated parents have deposited their eggs onto the plants. An analysis of transcript levels of defence genes revealed that plants with eggs from antibiotics-treated females show lower expression levels of several phytopathogen-responsive genes than plants with control eggs. Our study indicates that microbes are relevant “hidden players” in the interaction between plants and insect eggs.

Keywords: *Pieris brassicae*, *Brassica nigra*, *Arabidopsis thaliana*, bacteria, plant defence, insect eggs, oviposition

CO134

SYMBIOSE-MEDIATED ACONITINE RESISTANCE IN THE LEAF BEETLES *GALERUCA LATICOLLIS*

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During their evolution, plants have developed a variety of morphological and biochemical traits to fend off herbivores such as insects. The production of secondary metabolites is one of the most important plant biochemical defense. In our study system, *Aconitum napellus* (Ranunculales, Ranunculaceae) produces alkaloids, such as aconitine and nepalin, which are extremely toxic to animals. Despite their high toxicity, the leaf beetle *Galeruca laticollis* (Coleoptera, Chrysomelidae) is able to feed and complete its life cycle on this plant. In this study, we investigate the possible involvement of bacteria in this resistance phenotype. Strains isolated on different cultural media were assessed for their ability to *in vitro* degrade aconitine using HPLC. This indicated a strain of Pseudomonas as the most efficient degrader among our bacterial collection. In order to confirm these results *in vivo*, we used *Drosophila melanogaster* larvae as a heterologous model-host for *Pseudomonas sp.* After verifying the ability of our bacterium to colonise *D. melanogaster's* larval gut, an experiment in which the larvae were challenged with aconitine in their growth media was carried out. The results showed that the larvae colonised by Pseudomonas had a significant increase of their survival rate compared to non-colonised ones. These data clearly confirm the key role played by *Pseudomonas sp.* in degrading aconitine within its host's gut, thus helping it overcome plant defences. A genomic study of the bacterial ability to degrade aconitine identified 80 genes potentially involved in the metabolism of aromatic compounds. Out of these genes, 25 were differentially expressed when the bacterium was grown in presence of aconitine. The obtained results provide further evidence regarding the mechanisms by which insects are able to resist toxic molecules (plant secondary metabolites or some pesticides) through the evolution of beneficial symbioses with bacteria.

Keywords: Insect-plant interaction, symbiosis, Chrysomelidae, resistance to plant toxic metabolites

CO135

VIRUS-INDUCED CHANGES IN PLANT-INSECT INTERACTIONS IN THE GENUS *SOLANUM*

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The genus *Solanum* contains many important plants, including crops such as potato, tomato and aubergine as well as several popular ornamental varieties including the potato tree, Solanum crispum. In addition to their economic importance and popularity with gardeners, plants such as tomato can also be used as model systems. This project is using plants in the genus Solanum to further understand the interactions between plants, insects and plant viruses to inform and benefit amateur gardeners and the horticultural industry. The interactions between plants and associated insects are complex and often mediated by volatiles. Infection by viruses alters plant volatile signatures and consequently affects how insects interact with plants. Previous work at the University of Cambridge showed that bumblebees (*Bombus terrestris*) are more attracted to tomato plants (*Solanum lycopersicum*) infected with Cucumber mosaic virus (CMV) than to uninfected plants (Groen *et al.*, 2016). To explore the mechanisms behind the emission of bee-perceived volatiles by CMV infected tomato plants, bumblebee preference tests and volatile capture analysed by gas-chromatography coupled mass-spectrometry have been used to investigate the role of plant defence pathways and viral silencing proteins in mediating emission of bee-perceivable plant volatiles. I also plan to investigate the generality of the effect of CMV on plant-insect interaction previously seen in tomato by testing whether plant viruses with different modes of transmission cause similar effects on plant-pollinator interactions and undertaking field trials to investigate whether virus infected plants also attract bees outside of the lab or glasshouse situation.

Keywords: Bombus, Bumblebee, tomato, pollination, Multitrophic interactions, chemical ecology, cucumber mosaic virus, plant disease

Molecular Basis of Insect Communication

CO136

ELEVATED CO2 CONCENTRATIONS IMPACT THE SEMIOCHEMISTRY OF APHID HONEYDEW WITHOUT CASCADE EFFECT ON AN APHID PREDATOR

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Honeydew is now considered a cornerstone of the interactions between aphids and their natural enemies. Its composition is impacted by the aphid host plant species and associated phloem sap. Bacteria activity occurring in the aphid honeydew typically results in the release of volatile organic

compounds (VOCs) that are used by aphid natural enemies for prey location. Because atmospheric carbon dioxide (CO_2) concentration directly impacts plants physiology, we raise the hypothesis that elevated CO_2 concentrations impact the quantity of honeydew produced by aphids, as well as the diversity and quantity of honeydew VOCs, with cascade effects on the foraging behavior of aphid natural enemies. Using solid-phase microextraction, we quantified the VOCs emitted by honeydew from pea aphids (*Acyrthosiphon pisum* Harris) reared either under 450±50 ppm of CO_2 (aCO_2) or 800±50 ppm of CO_2 (eCO_2). While the total amount of honeydew (honeydew release by 190±50 individus in both conditions) is not impacted by the CO_2 concentration, we found qualitative and quantitative differences in the semiochemistry of aphid honeydew between CO_2 conditions. Three VOCs were not found in the honeydew of eCO_2 aphids: 3-methyl-2-buten-1-ol, 2-methyl-1-butanol and isobutanol. However, no difference was observed in the searching and oviposition behavior of hoverfly (*Episyrphus balteatus* De Geer) females exposed to infested plants reared under both CO_2 conditions, in a dual choice bioassay. The present work focuses on one particular aspect of atmospheric changes and should be extended to other abiotic parameters, such as temperature.

Keywords: Climate change, Carbon dioxide, *Acyrthosiphon pisum*, *Episyrphus balteatus*, Searching behavior, Honeydew production, Volatile Organic Compounds

CO137

FUNCTIONAL CHARACTERIZATION OF OLFACTORY RECEPTORS FROM *SPODOPTERA LITTORALIS* CATERPILLARS THROUGH HETEROLOGOUS EXPRESSION IN XENOPUS OOCYTES

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The Lepidoptera order groups many voracious herbivores of strong economic impact on plant production. Because their behaviours are in large part driven by odorants, the olfactory system appears as a target of choice to control pest populations. Insect olfactory receptors (ORs) are at the core of the olfactory detection process. However, only a few ORs have been functionally characterized and, outside the two dipteran models *Drosophila melanogaster* and *Anopheles gambiae*, we lack a complete view of how a given species uses its OR repertoire to efficiently detect its olfactory environment. Recently, we have functionally characterized the first large OR repertoire in a Lepidoptera, the cotton leafworm *Spodoptera littoralis*, using the “empty neuron” system, that is heterologous expression in Drosophila olfactory neurons coupled to single-sensillum recordings (de Fouchier *et al.* 2017). However, thanks to the recent annotation of ORs in the genome of *S. littoralis*, we estimate that the deorphanized ORs only represent one third of the total OR repertoire of this species. In this study, we have selected ORs expressed in *S. littoralis* caterpillars to characterize their function (*i.e.* determining their odorant ligands). We are developing an automated *in vitro* screening system allowing a high-throughput screening of OR-expressing Xenopus oocytes using two-electrode voltage clamp electrophysiology. Whereas most semiochemical-based pest control strategies currently focus on adults (pheromone traps, mating disruption,…), functional studies of caterpillar ORs will open new routes to target directly the herbivorous stage.

Keywords: *Spodoptera littoralis*, caterpillars, odorant receptors, Xenopus oocytes, two-electrode voltage-clamp.

CO138

THE ROLE OF CUTICULAR HYDROCARBONS IN MATE RECOGNITION IN *DROSOPHILA SUZUKII*

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Cuticular hydrocarbons (CHCs) play a central role in the chemical communication of many insects. In Diptera, CHCs have diverse functions, including in aggregation and mate and species recognition. Numerous factors impact CHC composition, including age, sex, maturation, interaction with the opposite sex, temperature, food, and hormones. *Drosophila suzukii*, the spotted wing Drosophila, is a recently introduced invasive pest insect species. Despite its economic importance, very little is known about chemical communication and the possible role of CHCs therein. We have used gas chromatography mass spectrometry to identify 60 CHCs in *Drosophila suzukii*. We demonstrate that age (maturation) is the key factor driving changes in the CHC profiles, while sex and interactions with the opposite sex are not. We tested the effect on courtship behaviour and mating of six selected CHCs, and demonstrate that four of the major CHC peaks with a chain length of 23 carbons, namely 9-tricosene (9-C23:1), 7-tricosene (7-C23:1), 5-tricosene (5-C23:1) and tricosane (n-C23), negatively regulate courtship and mating. We then go on to show that this effect on courtship and mating is likely caused by altering the natural ratios in which these hydrocarbons occur in *Drosophila suzukii* and subsequent disruption of mate recognition. We propose that a detailed understanding of chemical communication in *Drosophila suzukii* could lead to disruption strategies that can be part of integrated management of this important pest.

Keywords: *Drosophila suzukii*, cuticular hydrocarbons, mate recognition, chemical communication

CO139

ROLE OF THE TASTE IN HOST PLANT ACCEPTANCE BY ADULT FEMALES AND LARVAE OF PAPILIO HOSPITON GÉNÉ

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Lepidopterans represent a suitable model to study the relationship between sensory input and behavioral output, both in the identification of suitable oviposition sites by adult females and potential food sources by the larvae. In fact host specificity is determined not only by female oviposition preferences, but also by larval food acceptance. To verify whether peripheral taste sensitivity plays a role in the presence or absence of a positive relationship between oviposition preference and larval performance we selected *Papilio hospiton* Géné, an butterfly endemic of island of Sardinia that uses mainly plants of the Apiaceae family as hosts. Ferula communis is the host plant accepted by both ovipositing females and feeding larvae, *Seseli tortuosum* is accepted by females but not by larvae and *Foeniculum vulgare* is accepted by neither. Aim of this work was to investigate whether the balance between positive and negative gustatory inputs differed enough, between the two stages of the insect life cycle, to justify acceptance or rejection of the tested plants species as a possible host plant. To this end: a) we recorded and cross-compared the spike activity of larval and adult gustatory receptor neurons (GRNs) to taste stimuli, b) we evaluated the discriminating capability between saps and which neural code/s is/are used. The results show that: a) the spike responses of tarsal GRNs of adult females to Ferula and Seseli are not different from each other, but both differ from Fennel; b) larval L-lat GRN shows a higher activity in response to Seseli and Fennel than Ferula, while the opposite occurs for phagostimulant neurons and larvae discriminate among saps by means of an ensemble code; c) the number of eggs laid on Ferula an Seseli is the same, but larval growth performance is better on Ferula than on Seseli; no data are available for Fennel.

Keywords: Performance-preference hypothesis, ovipositions, food acceptance, chemoreception, Papilionidae

CO140

MOLECULAR BASIS OF LEPIDOPTERA CHEMICAL COMMUNICATION: FUNCTIONAL STUDIES OF ODORANT RECEPTORS FROM AN HERBIVOROUS SPECIES

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Today, entomology is facing the new era of high-throughput sequencing, with cumulative transcriptomics and genomics data available on a diversity of model and non-model species. Challenges are to exploit this diversity and to develop functional tools, especially in non-model species. Using the crop pest moth *Spodoptera littoralis* (Lepidoptera; Noctuidae) as a model species in chemical ecology, we developed such tools for the study of odorant receptors (ORs). These ORs are transmembrane proteins expressed in olfactory sensory neurons, and they are at the core of odorant detection. Each species has evolved a unique repertoire of ORs whose functional properties are expected to meet its ecological needs, though little is known about the molecular basis of olfaction outside Diptera. Using transcriptomics and genomics, we identified the complete set of ORs in *S. littoralis*. Heterologous expression in Drosophila antennae coupled to electrophysiology was used to functionally characterize these receptors. As a complementary tool, we established genome editing in this species via CRISPR/Cas9 to knock-out ORs. We report a pioneer functional analysis of a large array of ORs in a Lepidoptera. A phylogenetic analysis highlighted a basic conservation of function within the receptor repertoire of Lepidoptera, across the different major clades. We also demonstrate that the CRISPR/Cas9 system is highly efficient for genome editing in this crop pest. Our work opens new routes for gene function analysis and in fine the development of novel pest control strategies.

Keywords: Chemical communication, odorant receptor, functional characterization, CRISPR/Cas9, genome editing, Lepidoptera, *Spodoptera littoralis*

CO141

RESISTANCE TO GMOS AND PHEROMONE MEDIATED MATING IN THE FALL ARMYWORM

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The use of GM crops is now part of IPM programmes in different parts of the world and while they offer definite advantages they are not without potential problems. In Brazil the fall armyworm, *Spodoptera frugiperda* has developed resistance to Bt corn so we undertook experiments to determine if there were differences in pheromone mediated mating of resistant and susceptible strains. As Delisle and Vincent () had reported that insecticide resistant females of the oblique banded leaf roller spent less time calling and had lower pheromone gland titers that susceptible ones, which were considered costs associated with resistance. However, we found that resistant FAW females spent more time calling and had higher pheromone gland titers than their resistant counterparts. Furthermore, field results indicate that there may be assortative mating. These findings will be discussed within the context season migration and how this may impact resistance management strategies.

Keywords: Fall armyworm, pheromone mediated mating, Bt corn, resistance to Bt

CO142

GENE EXPRESSION PATTERN OF *AEDES AEGYPTI* COURTSHIP BEHAVIOR: A CHEMOECOLGY POINT OF VIEW

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Aedes aegypti is the most synanthropic and anthropophilic species of the Culicidae, always cohabiting with humans and being an extremely opportunistic species. *Ae. aegypti* females also exhibit the so called “skip oviposition” behaviour, which comprises the distribution of the eggs at several breeding sites. This is a major characteristic that shapes the overall pattern of potential breeding sites distribution, leading to highly fragmented “breeding units”. An immediate consequence of this behaviour is that encounters between male and females tend to be unlikely in this scenario, thus, compromising mating processes. It is well known that mechanisms by which mosquitoes locate their human hosts, nectar sources, and oviposition sites, are primarily olfactory driven. We present here the overall changes in *Ae. aegypti* females gene expression in response to courtship triggered by the presence of conspecific and heterospecific (*Aedes albopictus*) males; and also the gene expression pattern related to mating with conspecific males. Using a gene ontology approach on analysing gene expression variation in virgin females *versus* conspecific contact females we could observe around 7 fold enrichment in regulated genes related to odorant binding proteins (OBPs). Nevertheless, genes related to OBPs were not found enriched in the heterospecific comparison with virgin females, showing that *Ae. albopictus* male are not capable of inducing a similar response on *Ae. aegypti* females. Interestingly, inseminated females presented an overall gene expression profile similar to the virgin ones. Conspecific contact with males by *Ae. aegypti* females is the major cue for eliciting a mating response and priming females for copulation. This is

mainly achieved by down regulation of OBPs related genes that switches off female host seeking behaviour. Innovative control approaches focusing on the semiochemical systems of mosquitoes could be used in the effort to disrupt undesirable host–insect interaction in order to reduce the transmission of arboviruses.

Keywords: *Aedes aegypti*, courtship, odorant binding proteins

CO143

THE SISTER-SPECIES APPROACH IN ENTOMOLOGY AND ITS IMPORTANCE IN DISENTANGLING GENOMICS, PHYSIOLOGY AND ECOLOGY OF SPECIES

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Current entomology utilizes a large array of analytical tools taken from different disciplines such as ecology, neurobiology, microbiology, genetics, and genomics; this interdisciplinary approach can generate robust knowledge, clarifying the biology of insect species. There is however one crucial aspect often neglected: the comparison between an insect and its closest related sister-species *i.e.* the closest species or group of species on a phylogenetic tree. In the absence of a sister-species, comparisons cannot discriminate characters that are unique to the pest from those shared with other members of its clade. While the availability of a sister-species makes it possible to polarize characters into the phylogeny, (1) allowing the identification of unique chemosensory genes useful for chemical ecological applications, (2) pin-pointing the estimate age of speciation and evolutionary patterns for paleo-ecological reconstructions as well as (3) identifying with more accuracy typical physiological and behavioural characters. Due to budget and/or logistic reasons, this knowledge is too often impaired by a pest being compared only with distantly related species. Here we present the rationale behind the sister-species concept and provide some successful examples about the benefit of comparing an insect biology with that of the closer related species. We advocate that entomology would benefit from from adding to its repertoire the sister-species concept by modifying current research agendas.

Keywords: Sister species, phylogenetics, insect pest management

CO144

EXPLORING THE CHEMORECEPTOR REPERTOIRE AND THE ORCO-RNAI INDUCED BEHAVIORAL PHENOTYPES IN THE OLIVE FRUIT FLY, BACTROCERA OLEAE (DIPTERA: TEPHRITIDAE)

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The olive fruit fly (*Bactrocera oleae*) is the most destructive pest of the olive tree worldwide causing significant production losses and olive fruit impoverishment. The need to diminish conventional chemical approaches and develop efficient, specific control methods on the olive fly leads in the study of biological systems related to its harmful effect. Therefore, exploring the molecular basis of its chemoreception system will demonstrate the respective molecular targets that determine species-specific features on reproductive and sexual behavior, as well as host-plant recognition. To improve knowledge of olfactory perception and taste, we identified the complete olfactory receptor (OR) and gustatory receptor (GR) gene repertoires on the scaffolds of *B. oleae* genome. Gene structure, transmembrane helix prediction, and basic phylogenetic clustering were examined. Further interspecies comparisons were performed based on multiple sequence alignment of the entire OR protein sequence, to interpret similarities of orthologous copies, divergence or species-specific expansions among relative species. Furthermore, transient gene silencing of the olfactory co-receptor Orco was performed through dsRNA injections. Observation of copulation and oviposition rates demonstrated that silencing Orco plays a crucial role in the reproductive behavior of the olive fly, since pre- and/or post- mating processes were affected. These observed behavioral changes render this gene a potential target for the improvement of the olive fruit fly population control techniques. Screening and deorphanization of the chemoreceptors in the future, although still remain a challenging task, would allow the discovery of novel odorant-OR interactions to be tested towards the improvement and specialization of the olive fly control strategies.

Keywords: olfactory system, gustatory system, genomics, RNAi, oviposition, reproduction

Session 5.
Ecology and Toxicology of Insecticides

Insecticide Resistance: Evolution & Selection

CO145

THE MOLECULAR INNOVATIONS UNDERLYING RESISTANCE TO NATURAL AND SYNTHETIC XENOBIOTICS IN THE APHID *MYZUS PERSICAE*

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The process by which genetic novelty is created and drives the evolution of key innovations required for ecological adaptation is still relatively poorly understood. In this talk I will outline recent work investigating the mutational events driving host-range expansion and xenobiotic resistance in the peach potato aphid, *Myzus persicae*, the most economically important aphid pest worldwide. *M. persicae* is globally distributed and highly polyphagous with a host range of over 400 species including many important crop plants. Relatively recently this species host-shifted to tobacco, and we have previously demonstrated that the constitutive overexpression of a cytochrome P450, CYP6CY3, allows tobacco-adapted races of *M. persicae* to efficiently detoxify nicotine and has preadapted them to resist neonicotinoid insecticides. Our recent work has employed a suite of genomic, transcriptomic and post-genomic functional approaches to understand how genetic novelty was created and fuelled key innovations required for *M. persicae* to exploit a new ecological niche.

Keywords: Aphid, resistance, *Myzus persicae*

CO146

RESISTANCE STATUS OF *BEMISIA TABACI* TO NOVEL COMPOUNDS IN SPAIN

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Bemisia tabaci has readily developed resistance to numerous insecticide classes. Novel compounds such as diamides and ketoenols have been recently proposed for an effective control of this problematic pest. Cyantraniliprole is a novel anthranilic diamide insecticide. This new compound shows activity on a cross-spectrum of pests by activating ryanodine receptors. Spiromesifen and spirotetramat are novel insecticides belonging to the new chemical class of tetronic and tetramic acid derivatives, also known as ketoenols. They have a new mode of action, interfering with lipid biosynthesis. Toxicity to these compounds were determined for lab and field populations in the Mediterranean area in order to clarify the resistance status and cross-resistance patterns.

Keywords: *Bemisia tabaci*, whitefly, cyantraniliprole, spiromesifen, diamide, ketoenol

CO147

IDENTIFYING GENOMIC CHANGES ASSOCIATED WITH INSECTICIDE RESISTANCE IN THE DENGUE MOSQUITO *AEDES AEGYPTI* BY NEXT-GENERATION SEQUENCING

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Insecticide resistance threatens the control of mosquitoes transmitting arboviral diseases worldwide. Although alternative vector control tools are being developed, their global implementation will require some time and managing resistance to the few available chemical insecticides is crucial for sustaining vector control efforts for the next decades. In mosquitoes, insecticide resistance is mainly the consequence of modifications of the proteins targeted by insecticides (target-site mutations) and of the biodegradation of insecticides by detoxification enzymes (metabolic resistance). Target-site mutations are relatively well characterized and easy to monitor using molecular diagnostic tools, while the genetic factors controlling metabolic resistance are poorly characterized impeding their monitoring in natural mosquito populations. In this context, we adopted an integrative approach combining experimental evolution, quantitative genetics and next-generation sequencing to identify novel genetic markers of insecticide resistance in the dengue mosquito *Aedes aegypti*. This study identified copy number variations and non-synonymous polymorphisms in gene encoding detoxification enzymes specifically associated with resistance to different insecticide families. These results paves the way to the development of novel diagnostic tools able to concomitantly track the whole range of insecticide resistance mechanisms in order to improve resistance management.

Keywords: Mosquitoes, Insecticide Resistance, Experimental evolution, Next-Generation Sequencing

CO148

DISSECTING INSECTICIDE RESISTANCE VIA GENETIC MANIPULATION AND GENOME MODIFICATION IN DROSOPHILA

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To date, control of insect populations largely relies on the use of insecticides. However, agricultural pest and disease vector populations alike, rapidly develop resistance to several insecticide classes. Our research aims at the investigation of the mechanisms inducing resistant phenotypes using a variety of experimental approaches; among these, genetic transformation and genome modification in model species like Drosophila has played a pivotal role in the understanding of the role of individual alleles and the determination of specific insecticides' mode of action in several resistance cases. Certain examples include the investigation of the contribution of specific mutations in target-site resistance phenotypes (in genes like chitin synthase, ryanodine receptor and voltage-gated sodium channels) using a reverse genetics approach enabled genome modification by CRISPR/Cas9 coupled with homologous recombination. Furthermore, the tissue-specific heterologous overexpression of candidate detoxification genes, facilitated by GAL4/UAS mediated genetic transformation, is invaluable for the validation of particular genes implied in resistance. The versatility of these tools is demonstrated by the ability to devise *ad hoc* strategies in order to test hypotheses where the emergence of resistance may involve several different mechanisms. Such cases may be dissected in-depth with the combined use of genome modification, ectopic expression and/or knock-down of endogenous genes (via RNAi) in the same genetic context, using the unique available genetic toolkit and standard Drosophila genetics. Despite certain limitations, this approach greatly enhances our ability to investigate insecticide resistance in a fashion complementary to standard pipelines investigating resistance in the field and classical forward genetics. A number of successful (and some perhaps not-so-successful) cases currently under investigation will be discussed in detail.

Keywords: Insectidide Resistance, Drosophila genetic manipulation, CRISPR/Cas9

CO149

MOLECULAR CHARACTERISATION OF PHOSPHINE RESISTANCE USING RNA-SEQ IN TURKISH POPULATIONS OF *SITOPHILUS SPP*

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The development of strong phosphine resistance in stored product pests threatens the effective use of this important fumigant. In Turkey, there have been reports that strong resistance is developing, but not much information has been published. In this study we collected strains of *S. oryzae* and *S. granarius* from storages across Turkey and determined their phenotype and performed RNA sequencing using next-generation sequencing methods, *i.e.* RNAseq on the Illumina Miseq platform. This allowed us to determine and compare the resistance gene sequences to published phosphine resistance genes. For *S. oryzae*, 28 populations from 11 provinces were collected and 11 of these were determined to be resistant by bioassay. Of these strains, 3 resistant and 2 susceptible were selected for RNAseq analysis. The results showed that there were several resistance variants in the *rph2* gene, dihydrolipoamide dehydrogenase (*DLD*), that have not been previously reported. These variants (P48S, K141Q, K141E) correspond to known amino acid positions in other species (*Tribolium casaneum* and *Rhyzopertha dominica*) but are not seen in previously reported Asian populations of *S. oryzae* from Australia, Vietnam and China. This indicates that resistance in Turkish populations are likely to have evolved locally and the different variants seen shows that the populations sequenced are separated and may not travel widely across the grain storage network. For *S. granarius*, 26 populations were collected from 9 provinces. Phenotype testing by bioassay only detected 5 populations that had phosphine resistance, with resistance factors of 3.52-5.26x. This indicated that these populations are mostly susceptible and the resistance found is only weak resistance caused by the rph1 gene only. This was confirmed by RNAseq analysis of 3 resistant and 2 susceptible populations. The transcriptome for *S. granarius* has not been previously reported, so we assembled a transcriptome from susceptible data and determined the sequence of the *DLD* gene within this transcriptome sequence. No resistance variants in DLD were able to be detected with RNAseq analysis. These results indicate that strong phosphine resistance is not in very high frequency in *S. oryzae* and not detected in *S. granarius* populations across Turkey generally. This means that these species should be able to be managed using protocols designed to delay resistance development. Also, it have been developed genetic markers for phosphine resistance alleles in *S. oryzae* and are using these to survey and monitor for resistance is these populations.

Keywords: *Sitophilus oryzae*, *Sitophilus granarius*, phosphine resistance, dld, new markers

CO150

MECHANISMS OF INSECTICIDE RESISTANCE IN *BEMISIA TABACI* WITH SPECIAL REFERENCE TO ACETYL-COA CARBOXYLASE INHIBITORS

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Attributes such as genetic adaptability, reproductive potential, virus-vectoring capacity and extreme polyphagy conspire to make *Bemisia tabaci* (Hemiptera: Aleyrodidae) one of the world's most invasive and destructive insect pests. These traits also underpin its propensity to develop resistance to insecticides. Aside from its practical importance, research to diagnose, characterise and manage resistance in *B. tabaci* is shedding light on fundamental aspects of evolution and toxicology including gene expression, adaptive convergence, target-site pharmacology and the role of arrhenotoky in accelerating the selection of favoured traits. As a consequence of extensive exposure to insecticides on several crops including cotton, vegetables and ornamentals, *B. tabaci* has developed resistance to most of the chemical groups available for its control. Insecticide resistance patterns in *B. tabaci* are complex, and sometimes cross-resistance between chemical classes renders it difficult to implement resistance management strategies based on different modes of action. One of the latest classes of insecticides globally introduced for whitefly control are spirocyclic tetronic and tetramic acids such as spiromesifen acting on acetyl-CoA carboxylase, catalysing the rate limiting step in the biosynthesis of fatty acids. We have recently detected first cases of resistance to spirocyclic ketoenols in *B. tabaci* exceeding resistance ratios of 1000-fold when compared to a

susceptible reference strain. A detailed biochemical and molecular analysis revealed interesting insights into a yet undescribed mechanism of resistance.

CO151

FUNCTIONAL CHARACTERIZATION OF INSECTICIDE RESISTANCE AND THE VALUE OF MOLECULAR DIAGNOSTICS FOR INSECTICIDE RESISTANCE MANAGEMENT

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The prevention of vector borne diseases, as well as the protection of agricultural production, are best achieved by controlling the insect populations; a process that largely relies on the use of insecticides. However, some of the most important agricultural pests, as well as disease vectors worldwide, display an intriguing ability to develop resistance to multiple insecticides. Our research aims at the investigation of the mechanisms inducing resistant phenotypes. This research is facilitated by the use of a variety of experimental approaches, including bioassays, transcriptomic and proteomic analyses, functional characterization of recombinant enzymes *in vitro* and *in vivo*, immunohistochemistry – based dissection of detoxification physiology, genetic transformation and genome modification (GAL4/UAS and CRISPR/Cas9 in models like Drosophila as well as in non-model species). Our main focus is the in-depth understanding of the role of individual genes and pathways that co-evolve to produce striking resistance cases, in terms of both intensity and spectrum, primarily in a subset of insect species that are extremely difficult to control. Some examples of our ongoing studies where we have gained certain insight on such resistance cases will be presented. Among our research outcomes, an important milestone is the identification of molecular resistance markers enabling the subsequent development of modern molecular diagnostic platforms. Such molecular diagnostic tools greatly enhance our ability to monitor population dynamics and evolution of insecticide resistance in the field. But what is the real value of the molecular markers and associated modern diagnostics *versus* classical bioassays, for the implementation of evidence – based Insecticide Resistance Management strategies?

Keywords: Insecticide Resistance, IRM, molecular diagnostics

CO152

RESISTANCE TO ARTIFICIAL INSECTICIDES IN APHIDS AS A CONSEQUENCE OF LONG-TERM NATURAL EVOLUTION PRECEDING THE EMERGENCE OF INSECTICIDES

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Appearance of insecticide resistance in phytophagous insects is usually considered as a result of the strong driving selection occurring into environment which is regularly exposed to pesticides. It is known that insecticide resistance in insects is a result of increasing the basal level of expression or copy numbers of detoxification enzyme genes. However, despite the fact that the majority of resistant aphid clones belong to generalists, the link between polyphagy and the ability to display insecticide resistance has not been traced, even though polyphagy in aphids is an extremely rare phenomenon. According to our hypothesis, aphids which have adapted to feeding on an enormous number of different host-plants, including those containing defensive secondary metabolites, must be pre-adapted to respond to exposure to artificial insecticides with the activation of on-the-spot resistance mechanisms without the need to go through rigorous natural selection. The second question to answer was whether the feeding on the host-plant producing toxic secondary metabolites can modify some patterns of insecticide resistance in polyphagous aphids. We studied *Myzus persicae* (Sulzer) and *Aphis fabae* Scopoli, which are extremely wide generalists. The offspring of one female were divided into parthenogenetic clones on different host plants which constitutively differed in the composition of secondary metabolites. After one month of isolation on the host-plant, aphids from different clones were compared using the parameters: surviving under exposure of neonicotinoids, activity of Esterase and CYP450 enzymes, the level of expression of CYP4 and CYP6 genes. The number and variability of *CYP4*, *CYP6* and *Esterase genes* in the aphid genome were also examined. We found out that genetically identical clones of aphids significantly differed in all those parameters. A month feeding on a particular host-plant predictably modified the ability to survive under insecticidal treatment that was provided with increasing of Esterase and CYP450 enzymes production.

Keywords: Aphids, insecticide resistance, Myzus persicae, Aphis fabae

CO153

SUPERMUTANT: THE CASE OF GRAIN APHID (*SITOBION AVENAE*) RESISTANCE TO PYRETHROID INSECTICIDES

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Pesticide resistance facilitates adaptation and survival through a range of different mechanisms that reduce or diminish the effectiveness of an active ingredient, for example by preventing its mode of action at the target site. Resistance to pyrethroid insecticides detected in 2014 in *Sitobion avenae* (Fabricius) (Hempitera: Aphididae), a prolific cereal pest and vector of Barley Yellow Dwarf Virus (BYDV), presents a serious challenge to cereal growers. Resistance has been attributed to the classic target site mutation (L1014F) known as Knock Down Resistance (kdr), found on one allele of the aphid sodium channel gene. Resistance screening and microsatellite genotyping over a three year period in Ireland reveals that kdr-heterozygote aphids occur extensively in cereal fields and adjoining grass verges, with resistance predominantly in one SA3 superclone. Furthermore, a second resistance mechanism in the form of pesticide detoxification has been detected in some SA3 clones, likely linked to enhanced cytochrome P450 monoooxygenase activity. Resistance mutations are frequently believed to carry fitness penalties. However, our research provides evidence that whilst the heterozygote kdr SA3 *S. avenae* superclone can survive pyrethroid exposure up to twice the normal field application rate, it continues to be able to reproduce asexually at rates comparable to fully susceptible individuals. Furthermore, under laboratory conditions sexual capacity is retained in the SA3 superclone, with the observation of oviparous morphs, creating the possibility of gene-flow through a wide range of (androcyclic, holocyclic and intermediate) breeding systems, and creation of kdr-homozygote offspring through sexual crossing between kdr-heterozygotes if heterozygote resistant male aphids are produced. These findings have serious implications for resistance management and the future efficacy of pyrethroid insecticides in controlling grain aphids and the associated risk of BYDV transmission in crops.

Keywords: Fitness penalty, grain aphid, knock down resistance, pesticide resistance, pyrethroid detoxification, resistance gene-flow, SA3 superclone, sexual reproduction, *Sitobion avenae*

Insecticide Resistance: Detection & Monitoring

CO154

THE RELATIVE CONTRIBUTION OF TARGET-SITE MUTATIONS IN COMPLEX ACARICIDE RESISTANT PHENOTYPES IN *TETRANYCHUS URTICAE*

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The molecular mechanisms underlying insecticide and acaricide resistance in insects and mites are often complex. Both target-site insensitivity and an increased metabolism, transport and excretion (or a combination thereof) can all contribute to the resistance levels observed in field populations. The relative contribution of target-site resistance mutations to the resistance phenotype has remained poorly understood in most arthropod pests. Here, we used marker-assisted backcrossing to create 34 congenic lines of the a polyphagous arthropod pest, the spider mite *Tetranychus urticae*. These lines share a common pesticide-susceptible genomic background, except for 10 loci that carry mutations (alone, or in combination in a few cases) that are associated with resistance to avermectins, pyrethroids, mite growth inhibitors, mitochondrial complex I (METI-I) and complex III (QoI) inhibitors. Toxicity tests revealed that mutations in the voltage-gated sodium channel, chitin synthase I, PSST subunit of the respiratory complex I and cytochrome b confer moderate to high levels of resistance and, when fixed in a population, these mutations alone can result in field failure of acaricide treatment. In contrast, the glutamate-gated chloride channels mutations do not lead to the high resistance levels that are often reported in abamectin resistant strains of *T. urticae*. Last, we used a complementary approach to evaluate the effect of a resistance mutation in Drosophila as a model organisms. We used the CRISPR-Cas9 genome editing tool to introduce the mutation conferring resistance to METI-I in the Drosophila PSST homologue. Overall, this study functionally validates reported target-site resistance mutations in *T. urticae*, by uncoupling them from additional mechanisms, allowing to finally investigate the strength of the conferred phenotype *in vivo*.

Keywords: Near-isogenic lines, NIL, target-site mutation, *Tetranychus urticae*, backcrossing 20 kDa subunit, METI-I, rotenone, Acari, Drosophila

CO155

USING CRISPR TO INVESTIGATE MECHANISMS OF NEONICOTINOID RESISTANCE IN *DROSOPHILA MELANOGASTER*

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Neurotoxic insecticides enter the insect body and encounter metabolic enzymes (*e.g.* cytochrome P450s and glutathione-s-transferases) during a journey that takes them to receptors in the brain. Movements between tissues are controlled by transporters, such as the ABC transporters. Appropriate mutations in genes encoding any of the proteins that interact with an insecticide could confer resistance. The potential for such resistances to evolve in the field will depend on many factors including the ratio of resistance conferred to fitness costs incurred. In our lab we have adopted a systems approach to understand the interaction between the neonicotinoid imidacloprid and each of these types of proteins. This has involved using CRISPR and controlled tissue-specific overexpression to manipulate genes, twin ion mass spectrometry to monitor levels of the insecticide and metabolites and a movement assay (the Wiggle Index) to assess the level of intoxication.

Keywords: Imidacloprid, nicotinic acetylcholinesterases, cytochrome P450s, glutathione-s-transferases, ABC transporters

CO156

MINING GENES INVOLVED IN INDOXACARB RESISTANCE OF *LOBESIA BOTRANA* (DENIS AND SCHIFFERMÜLLER) BY *DE NOVO* TRANSCRIPTOME ASSEMBLY AND DIFFERENTIAL EXPRESSION ANALYSIS

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Lobesia botrana (Denis and Schiffermüller) (Lepidoptera: Tortricidae) is one of the most important grapevine pests in Europe but, being a non-model organism, only limited genomic and transcriptomic resources are available for functional studies at the molecular level, such as those relevant to insecticide resistance and pest control. Hence, to gain insight into the mechanism of indoxacarb resistance, a blocker of insect voltage-gated sodium channels (NaV), we analysed the transcriptome and expression profile in 2nd instars of *L. botrana* from susceptible and field selected populations (LC50 resistance ratio 72). *De novo* transcriptome assembly using Trinity resulted in 141,581 isoforms clustered in 94,290 putative genes. The transcriptome completeness was supported by BUSCO: 92% of conserved orthologs (n=1,658) were retrieved as a complete sequence, 6.3% displayed fragmented ORFs, and only 1.7% were missing. 36,250 genes were preliminary annotated relaying on the longest isoform per gene, by running Annocript pipeline against non-redundant protein databases (Nr), gene ontology (GO), cluster of orthologous groups of proteins (COG), KEGG orthology (KO) and long non-coding RNAs (lncRNAs). Conditional Reciprocal Best BLAST analysis of protein isoforms performed on Lepidoptera proteomes identified putative orthologs of multigene family members potentially involved in metabolic resistance (61 cytochrome P450 monooxygenases, 25 glutathione S-transferases, 13 carboxylesterases, 25 UDP-glucuronosyltransferases) as well as alternatively spliced isoforms of the NaV gene. Among 263 upregulated and annotated genes in the resistant population, functional GO enrichment analysis revealed overrepresentation of terms for cytochrome P450, due to up-regulation of CYP6B and CYP9A subfamily members as well as increased transcript level for UGT genes. Hydrolases were, on the contrary, overrepresented in 293 annotated genes, downregulated in the resistant population. These data tentatively suggest the reduced susceptibility to indoxacarb might be related to an increase of Phase I and II detoxification along with reduced bioactivation of the insecticide.

Keywords: *Lobesia botrana*, insecticide resistance, indoxacarb, transcriptome, differential expression analysis

CO157

MECHANISMS OF RESISTANCE TO DELTAMETHRIN IN ISOFEMALE LINES OF THE MOSQUITO *AEDES AEGYPTI* FROM FRENCH GUIANA

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Aedes aegypti is vector of dengue, chikungunya and Zika viruses in urban area of French Guiana, a French territory in South America. Deltamethrin, a pyrethroid insecticide, remains the sole insecticide molecules authorized for adult control in the European Union to which French Guiana belongs. However, resistance to deltamethrin has been monitored in several populations of *Aedes aegypti* from French Guiana and revealed an extreme resistance to this molecule. In order to better understand the mechanisms of resistance, four isofemale strains have been successfully isolated locally, with similar genetic backgrounds but with different resistance spectra to deltamethrin (pyrethroid). We performed enzymatic activity assays of detoxification enzymes involved in insecticide resistance. We also monitored mutations located at the position 1016 and 1534 of the sodium voltage-gated channel gene in these populations by Taqman Allelic Discrimination Assays. These mutations were already linked to pyrethroid resistance in *Aedes aegypti* populations from Latin America. Resistance to other insecticide families (organophosphates and carbamates) was also monitored in our strains that exhibited different level of resistance to deltamethrin. Our study revealed different combinations of resistance mechanisms in our lab strains: metabolic resistance or resistance associated to a mutation of the sodium voltage-gated channel gene. The metabolic resistant strains have an increase esterase activity while other strains exhibit mutation at the position 1016 of the sodium voltage-gated channel gene. Moreover, selection to deltamethrin led to an increase resistance to other class of insecticides suggesting inherited predisposition to multiple resistance. The results obtained on these laboratory lines are essential for understanding the resistance to deltamethrin as well as multiple resistance phenomenon in the natural populations of *Ae. aegypti* of French Guiana.

Keywords: *Aedes aegypti*, isofemale strains, insecticide resistance, multiple resistance

CO158

INSECTICIDE RESISTANCE IN UK PESTS: THE GOOD, THE BAD AND THE UGLY

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The evolution and spread of insecticide resistance in agricultural and horticultural pests continues to threaten our ability to protect our crops and imposes increased selection pressures on the active compounds that remain in our armoury. European Legislation, making restrictions on the use of neonicotinoids as seed treatments, is only exacerbating this situation. As a result, continued monitoring of any changes in the resistance profile of insect pest populations is crucial if we are to communicate relevant, up-to-date information to agronomists, growers and government regulators on which insecticides will currently be effective and which will not. This presentation will summarise the insecticide resistance profiles currently seen in a range of important UK pests (aphids, beetles, moths and thrips); information gained through work collaboratively funded by Agrochemical Companies, Commodity Boards and Agronomy Companies.

Keywords: Insecticide resistance, insect pests, insecticides

CO159

WESTERN CORN ROOTWORM RESISTANCE TO BT MAIZE WITHIN AGRICULTURAL LANDSCAPES

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The western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae), is a serious pest of maize in the United States and is currently managed by planting transgenic maize that produces insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (Bt). In some portions of the United States, western corn rootworm has evolved widespread resistance to transgenic maize producing Bt toxin Cry3Bb1. Resistance to Cry3Bb1

has been found to extend to Bt toxins mCry3A and eCry3.1Ab through cross-resistance. More recently, evidence of emerging resistance to Cry34/35Ab1 maize by western corn rootworm also has been found for some field populations, which means that all of the four Bt traits currently available for management of western corn rootworm with transgenic maize have been compromised by resistance. This presentation will address what is known about the geographic distribution of resistance based on sampling of field populations and subsequent bioassays. Additionally, data will be presented on factors contributing to the evolution of resistance in field populations. Management of Bt resistance by western corn rootworm has focused on the refuge strategy in conjunction with pyramiding of multiple Bt toxins targeting rootworm. Delays in the evolution of resistance achieved by the refuge strategy are expected to be greater when resistance is inherited as a recessive trait and fitness costs are associated with resistance. Data from laboratory experiments using strains with field-derived resistance will be presented that quantify the inheritance and fitness costs of Bt resistance in western corn rootworm. The results of these study will be discussed in the context of applying insect resistance management and integrated pest management to delay the evolution of resistance by western corn rootworm and other agricultural pests managed with Bt crops.

Keywords: Bt maize, fitness costs, inheritance, insect resistance management, western corn rootworm

CO160

PYRETHROID SUSCEPTIBILITY STATUS OF *AEDES ALBOPICTUS* AND *CULEX PIPIENS* POPULATIONS ACROSS ITALY

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Invasive *Aedes albopictus* and indigenous *Culex pipiens* are the most abundant mosquito species in urban areas in Italy. Both species can create considerable nuisance to citizens, and may also transmit arboviruses such as Chikungunya, Dengue (*Ae. albopictus*) and West-Nile (*Cx. pipiens*). Although insecticides represent a fundamental tool to reduce mosquito abundance and limit disease transmission, little is known on insecticide susceptibility of these species. Previous studies allowed to highlight resistance to pyrethroid insecticides commonly used to reduce adult abundance in *Ae. albopictus* populations from Ferrara (Emilia-Romagna) and Bari (Puglia) provinces. In the present study, we focused on populations of *Ae. albopictus* from Emilia-Romagna, Lazio and Calabria, the regions where the species has been responsible for Chikungunya outbreaks both, in 2007 or in 2017. Furthermore, we carried out a first assessment of levels of susceptibility to pyrethroids in Italian populations of *Cx. pipiens*, in order to compare levels of resistance amongst the two species. Insecticide resistance tests were performed following WHO protocols by exposing adult mosquitoes for 1 hour to permethrin, α-cypermethrin, deltamethrin and recording mortality at 24 hours after exposure. Our data confirm the presence of reduced susceptibility in some Italian populations of *Ae. albopictus* to permethrin. Moreover, most *Cx. pipiens* populations analysed exhibit a clearly lower susceptibility than sympatric *Ae. albopictus*, with mortality being in few sites below 30%. Results suggest that insecticide resistance phenotypes are present also in Italy, with important differences between species and sites. The lower levels of susceptibility recorded in *Cx. pipiens* populations could suggest that insecticide spraying is mostly affecting nocturnal *Cx. pipiens* rather than diurnal *Ae. albopictus*, against which they are targeted. Overall, results stress the need to carefully monitor insecticide resistance spread in Italy, also by means of molecular markers currently under investigation.

Keywords: Insecticide resistance, vector control, *Aedes albopictus*, *Culex pipiens*

CO161

FUNCTIONAL CHARACTERIZATION AND SUBSTRATE SCREENING OF UDP-GLYCOSYLTRANSFERASES OF THE TWO-SPOTTED SPIDER MITE TETRANYCHUS URTICAE

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The two-spotted spider mite *Tetranychus urticae* (Chelicerata: Acari) is an extreme generalist herbivore, able to feed on more than 1000 plant species. *T. urticae* is also the 'resistance champion' among arthropods, as it has the most documented instances of resistance to diverse pesticides. Uridine diphosphate (UDP)-glycosyltransferases (UGTs) catalyze the addition of UDP-sugars to small hydrophobic molecules, turning them into more water-soluble metabolites. Therefore, glycosylation by UGTs might play an important role in the detoxification of xenobiotics. *T. urticae* has 80 UGT genes, and most likely acquired these from bacteria through horizontal gene transfer. Out of these 80 UGT genes we identified a set of eight genes that were upregulated in mites upon plant adaptation and/or in mite strains highly resistant to certain acaricides. Next, we functionally expressed this set of *T. urticae* UGTs in *Escherichia coli* and recombinant enzymes were found to be catalytically active against model substrates. Upon initial enzyme characterization we performed a high-throughput substrate screening to investigate their potential to conjugate UDP-glucose to a diverse array of 41 acceptors comprising both plant metabolites and acaricides. Finally, nine enzyme-acceptor interactions were further studied more into detail by determining their kinetic parameters and determining their preferred UDP-donor substrate.

Keywords: Glycosyltransferase, horizontal gene transfer, pesticide resistance, plant adaptation

CO162

CHARACTERIZATION AND MONITORING OF TARGET-SITE RESISTANCE IN THE RYANODINE RECEPTOR OF THE TOMATO LEAFMINER, *TUTA ABSOLUTA*

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Tuta absoluta is an invasive species on the Eurasian continent and Africa. Native to South America, it arrived in Europe (Spain) 12 years ago. It has since then kept spreading, being reported now across Africa, the entire Mediterranean, the Middle East and South Asia. The control of *T. absoluta* relies an integrated pest management strategies of which modern chemistry such as the diamide insecticides form a key pillar. The efficacy of chemical control measures is threatened by resistance development and just as the pest itself, resistance mechanism(s) may spread fast. In this study we investigated the impact of mutations in the diamide target-site, the ryanodine receptor. Moreover we monitored the frequency of mutations in field populations across Europe and linked them to resistant phenotypes.

Keywords: *Tuta absoluta*, insecticide resistance, ryanodine receptor, target-site

Insecticide Mode of Action: Biological and Biochemical Aspects

CO163

OXIDATIVE AND ANTIOXİDANT EFFECTS OF JUGLONE ON THE GREATER WAX MOTH *GALLERIA MELLONELLA* L. (LEPIDOPTERA: PYRALIDAE)

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In this study aimed to investigate the effects of the oxidative and antioxidant effects of juglone (5-hydroxy-1,4-naphthoquinone) which is one of the secondary metabolites naturally synthesized in leaves and fruit of the walnut species in the genus Juglons and important phytochemical compound, on the greater wax moth *Galleria mellonella* larvae. *G. mellonella* is a model insect for ecotoxicological, ecophysiological and immunological investigations and a serious pest in wax. Investigation of all parameters assessed within the scope of the study was carried out on the larvae, the harmful stage of the pest. For this purpose, the insecticidal effect of the juglone was determined with probit analysis by applying technical juglone at different doses to the diets of the first instars of *G. mellonella*. Juglone showed insecticidal effect on the larval stage of the pest and lethal (LD99) and median lethal doses (LD50) were determined as 6.1 and 2.3mg/2g in diet, respectively. According to LD50 dose of juglone, the effective doses of 0.5, 1 and 2 mg in 2 g of diet were used for the ecotoxic analyses. Tissue homogenates of the last instars exposed to the effective juglone doses were used for antioxidant enzyme activities (SOD, CAT, GPx, and GST) and the amount of MDA. It was determined that antioxidant enzyme activities and the amount of MDA showed dose-dependent changes in tissue homogenates of last instars of *G. mellonella* treated with dietary juglone doses with respect to untreated larvae, in particular increases were detected at 1 mg dose of juglone. In conclusion, these study show that effective doses of juglone have ecotoxic and oxidative effects on the larval stage of pest and model organism *G. mellonella*.

Keywords: Juglone, *Galleria mellonella*, Antioxidant enzymes, Oxidative stress, Ecotoxicology, Naphthoquinone

CO164

NATURAL SUBSTANCES AS SOURCES FOR NEW INSECTICIDES

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Insect pests are a global issue affecting crops, forests and preserved products, with detrimental effects to human and animal health. In the past, their control mainly relied on synthetic insecticides, as organochlorine and organophosphate that actually are not environmentally sustainable and whose residues may affect human health. Thus, to control insect pests, alternative eco-friendly and safe products and methods are urgently needed. Recently, strong efforts are being made to identify and characterize natural products with insecticidal and/or repellent activity against insect pests. Those products, globally defined by the European Environment Protection Agency (EPA) as biopesticides, are a broad group that include botanicals (vegetal extracts), natural inert dusts as well as microorganism’s derived compounds. Some of these products are already largely utilized with success as bioinsecticides, but research is still ongoing on numerous other naturally occurring substances. It is the case of many botanicals (essential oils), vegetal, microbial, and animal derived substances, like peptide chains (proctolin, trypsin.) or the neurotoxic polypeptides derived from scorpions venom. Generally, the low toxicity, no residues in food, the high efficacy at low concentrations and the fact that they, theoretically, do not induce resistance in the treated insect populations, make the use of the new biopesticides highly promising. Currently, the relatively high cost and the variability of effectiveness make most biopesticides a niche product in the insecticide market. However, new formulation strategies are being evaluated and even if it is unlikely that biopesticides will completely replace chemical protection against pests within the next years, probably, the number of biopesticides-based products will increase, and the costs will fall guaranteeing an increasing place in the market for them for the near future.

Keywords: Biopesticides, botanicals, inert dusts, fungi, bacteria, peptide chains

CO165

POTENTIAL IMPLICATIONS OF INSECTICIDE-INDUCED HORMESIS FOR INSECTICIDE RESISTANCE, TOLERANCE, AND PRE-CONDITIONING

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Stress is generally perceived as harmful to organisms. However, it is now recognized that low amounts of stress, in any form (e.g. chemical, temperature, radiation, or nutritional stress), can have stimulatory effects on biological processes. In the same light, we now know that insecticides that are lethal or inhibitory to insects at high doses can stimulate them at certain low doses. The biphasic response to stress in the form of high-dose inhibition and low-dose stimulation is termed “hormesis”. The phenomenon has been seen in many insect taxa following mild exposure to stressors, including insecticides. Insecticide-induced hormesis in insects is most often observed as stimulated reproduction, although stimulatory effects on longevity, weight, and other physiological and behavioral processes have also been reported following insecticide exposure. Because insects in agro-ecosystems are often exposed to sub-lethal doses of insecticide that may very well be in the “hormetic zone”, the ramifications of insecticide-induced hormesis for crop protection may be significant. Using an aphid-imidacloprid model, we demonstrate that exposure to hormetic concentrations of insecticide that stimulate reproduction can prime insects to better withstand subsequent pesticide and nutritional stresses, although effects may be subtle over generations. We also show that insecticide-induced reproduction hormesis in aphids is accompanied by intermittent changes in expression of detoxification and stress-coping genes. Our results suggest that insecticide-induced hormesis may have implications for insecticide resistance, tolerance, and pre-conditioning.

Keywords: Hormesis, sublethal insecticide exposure, pest outbreaks, insecticide resistance, pre-conditioning

CO166

EXPRESSION AND MECHANISM OF TOXICITY OF A NOVEL MOSQUITOCIDAL CLOSTRIDIAL TOXIN

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Mosquito-borne diseases have been significantly impacted human civilization despite of centuries of intensive control effort. Diseases such as dengue, yellow fever, chikungunya or Zika which are transmitted most commonly by an infected Aedes female mosquito, filariasis and West Nile fever by Culex, and malaria by Anopheles mosquito species, still remain serious public health problems with constant threats of re-emergence. *Bacillus thuringiensis israelensis* and *Lysinibacillus sphaericus* formulations have become the predominant non-chemical means for Culex and Aedes mosquito control. However, new biological insecticides with different mode of actions are needed in order to broaden susceptibility range and avoid mosquito resistance. In our work, we compared the toxicities of two *Clostridium bifermentans* mosquitocidal strains and *Bacillus thuringiensis israelensis* against *Aedes aegypti*, *Anopheles gambiae* and *Anopheles stephensi*. The most susceptible mosquito species was *Anopheles gambiae* to *Clostridium bifermentans malaysia*. In order to find its toxic components, we sequenced the genome of the two *C. bifermentans* mosquitocidal strains, a non mosquitocidal *C. bifermentans* wild type strain and a non-toxic *C. bifermentans* malaysia mutant that we developed using gamma irradiation. Our sequencing data showed that *C. bifermentans* mosquitocidal strains contain a plasmid containing two toxin loci. The first one is composed by Cry16A, Cry17A and two hemolysins which was shown to be toxic to *Aedes aegypti*, but not to Anopheles mosquitoes. Interestingly, the second toxin locus has similarity to botulinum neurotoxin-like loci, which we renamed Clostridial Mosquitocidal Protein (CMP) locus. We produced different constructs encoding the proteins included in CMP locus and showed that they are responsible for Anopheles but not to Aedes toxicity. Finally we show that the CMP protein acts via a novel mechanism of action for mosquitocidal toxins.

Keywords: Anopheles. mosquitocidal, Clostridial, toxin, mechanism

CO167

NEW OPPORTUNITIES TO INVESTIGATE INSECTICIDE AND ACARICIDE CHLORIDE CHANNEL TARGETS THROUGH THEIR DIFFERENTIAL INTERACTIONS WITH DIVERSE CHEMICAL CLASSES

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The GABA- and glutamate-gated chloride channels are the major inhibitory neurotransmitter receptors of the brain, and are targeted by a diverse range of chemical classes including cyclodienes, phenylpyrazoles, isoxazolines, meta-diamides (GABA-Rs) and macrocyclic lactones (Glu-Rs). The evolution of resistance to these chemicals is an ever increasing problem for effective pest control and often arises due to the selection of by simple point mutations in these targets that disrupt the highly selective binding of these pesticides to the protein. Recent advances in the availability of sequence and molecular structure data for both GABA-R and Glu-R targets are being exploited to develop molecular models of how the different chemical groups interact with their target receptors. Comparative analysis of the functionality of known and novel resistance mutations in these targets, and the validity of our models, will be assessed by the expression of cloned GABA-R/Glu-R cDNAs in Xenopus oocytes and analysis of the modified receptors by two electrode voltage clamp methods. Electrophysiology testing of wild type and mutagenized GABA-R cDNAs demonstrates varying responses to GABA-R ligands such as GABA and phenylpyrazoles. The efficacy of ligand binding is being evaluated in this system and will be discussed in the context of pesticide selectivity and resistance.

Keywords: Pesticide, resistance, mutation, electrophysiology, GABA receptor

CO168

INHIBITION OF HISTONE ACETYLATION AND DNA METHYLATION NEGATIVELY AFFECTS LIFE HISTORY TRAITS OF THE PEA APHID (*ACYRTHOSIPHON PISUM*)

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Epigenetic mechanisms such as acetylation of histones and methylation of DNA play important roles in regulating gene expression in eukaryotes. These mechanisms are regulated by complex interplay among different enzymes. DNA methyltransferases (DNMTs) are mainly responsible for DNA methylation, whereas histone acetyltransferases (HATs) and histone deacetylases (HDACs) regulate histone modification. The acetylation of histones by HATs can increase accessibility of DNA to transcriptional factors and promote gene expression, whereas deacetylation process mediated by HDACs has the opposing activity. Inhibition of evolutionary conserved epigenetic mechanisms using specific inhibitors can negatively affect a range of life history traits of an organism. We investigated the fitness of harmful agricultural pest and model pea aphid (*Acyrthosiphon pisum* Harris) after chemically altering transcriptional activity of genes encoding components of HATs, HDACs and DNMTs. Specific inhibitors of both epigenetic mechanisms negatively affected *A. pisum* including its survival, reproduction and development. Remarkably, HDAC and HAT inhibitors severely compromised development of *A. pisum* with opposing effect on body weight. Observed fitness costs probably reflect significant changes in expression of several genes encoding HDACs and HATs after exposure to epigenetic inhibitors. Given that resistance to chemical insecticides develops rapidly in aphids, our study suggests that epigenetic apparatus holds the potential for discoveries of targets for novel insecticides or insect resistant crops.

Keywords: Epigenetic inhibitors, *Acyrthosiphon pisum*, histone acetylation, DNA methylation, fitness

CO169

INSECTICIDE MODE OF ACTION AND RESISTANCE MANAGEMENT: LESSONS LEARNED FROM THE CASE OF *TUTA ABSOLUTA*

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Insecticides are extensively used in protecting the crop production from insect pests worldwide. Numerus chemical compounds are currently available in the market that have been classified in different Mode of Action (MoA) Groups by Insecticide Resistance Action Committee (IRAC). Insecticides with different MoAs affect different key functions of the insects, thus controlling the pest population and the infestation levels in diverse ways. This is an essential knowledge, with significant practical projections, for implementing integrated resistance management tactics (IRM) in current pest management schemes. Within the frame of this presentation a brief overview on the diversity of the available MoAs will be presented. In order to highlight the importance of IRM and the rational use of the different MoAs the case of tomato leafminer *Tuta absoluta* will be used as an example; a novel pest for Europe that the past years has caused unprecedented crop losses. In part, the severity of pest status of *T. absoluta*, is associated with the capacity of the pest to rapidly develop resistance to different MoAs. Among the insecticides to be discussed here, the diamide insecticides flubendiamide and chlorantraniliprole exhibited the highest action against *T. absoluta* and subsequently the strongest reduction in the susceptibility levels. The resistance mechanisms and the potential reasons behind this major shift in the efficacy levels of diamides, at a global scale, were investigated. The lessons learned from managing *T. absoluta* and the implications in future insecticide resistance management tactics will be discussed.

Keywords: Mode of Action, resistance management, *Tuta absoluta*, diamides

CO170

LONG-TERM POPULATION SELECTIONS AND A CHROMOSOME-LEVEL ASSEMBLY REVEAL THE COMPLEX GENETIC ARCHITECTURE OF ACARICIDE RESISTANCE IN *TETRANYCHUS URTICAE*

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Spider mites are important crop pests with a remarkable evolutionary potential to adapt to xenobiotics such as plant secondary compounds and acaricides. Although crucial for devising efficient crop protection strategies, the mechanisms underlying rapid adaptation are not well understood. However, protocols for high resolution mapping of adaptation genes by bulk segregant analyses have now allowed to study resistance mechanisms free from prior hypothesis, with remarkable results. Next to the elucidation of a number of cases of monogenic (target-site) resistance, advanced genetic mapping now also allows to determine QTLs of complex cases of resistance. We report here on a proof of principle study using a chromosome level genome assembly.

Keywords: Resistance, genetic mapping, bulk segregant analysis, acaricides, *Tetranychus*

CO171

VOLTAGE-GATED SODIUM CHANNELS AS TARGETS FOR PYRETHROID INSECTICIDES

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The pyrethroids are a highly successful group of insecticides that have been used extensively for the control of arthropod pests of agricultural crops and vectors of animal and human disease. Unfortunately, their intensive use has led to the development of widespread resistance to these compounds in many species. Pyrethroids target the voltage-gated sodium channel of nerve membranes and, in many cases, resistance has been shown to result from specific amino acid substitutions within the channel pore that reduce pyrethroid sensitivity. This talk will review the current range of substitutions implicated in resistance and how a knowledge of these mutations has enhanced our understanding of the mode of action of these compounds through the modelling of putative binding site(s) within the channel protein and the identification of key residues involved in selectivity. The unusual and evolutionarily-unique structure of the voltage-gated sodium channel in aphids will also be discussed.

Keywords: Pyrethroids, sodium channel, insecticide resistance, aphids

Insecticide Toxicology and Non Target Effects

CO172

THE NON-TARGET EFFECTS OF MODERN INSECTICIDES ON NATURAL ENEMIES OF INSECT PESTS

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Combining selective insecticides and natural enemies of pestsis crucial for implementing sustainable integrated pest management packages. Pesticide risk assessment for natural enemies of insect pests inhabiting agroecosystemscan be challenging when (i) evaluating newer classes of pesticides that can cause sublethal effects rather than direct mortality, and when (ii) including other stressors such as high temperature regimes. Here, we present the results of several investigations aimed atdeepening the knowledge on behavioral and physiological sublethal effects of various modern insecticides toward parasitoids and predators of invasive insect pests.

Keywords: Biocontrol, biopesticide, ecotoxicology, risk assessment, sublethal effect

CO173

RECENT TRENDS IN INSECTICIDE TOXICOLOGY AND NON-TARGET EFFECTS WITH SPECIAL REFERENCE TO BEE POLLINATORS

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The current EU restrictions on the use of 3 neonicotinoids as seed treatments in crops that flower, were introduced in December 2013. This was to prevent a perceived risk to pollinators, especially bees and were initially for a two-year period. However, they are still in place and there is now a discussion on extending the ban to other crops that don't flower and don't attract bees. Potentially this could also lead to restrictions on other neonicotinoids and indeed to other insecticide classes. These policy decisions are often taken without due regard to risk *versus* hazard, the potential risk of alternatives or the variation in toxicity of different compounds within a chemical class. In this talk I will discuss the steps leading up to the current neonicotinoid restrictions and what has happened since, both in terms of published data and public/media debate. I will also cover the data we have on the toxicity of different neonicotinoids (and other pesticides) towards bees, especially how the ability of bees to metabolise insecticides can affect the toxicity.

Keywords: Neonicotinoids, bees, toxicity, policy

CO174

THE MOLECULAR IMPACTS OF CHRONIC PESTICIDE EXPOSURE ON BUMBLEBEE LEARNING AND MEMORY

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Insect pollinators are vital ecosystem service providers, contributing over $265 Billion dollars (USD) a year to the global economy. Estimates predict that 75% of the world's top 100 crops are insect pollinated in some form and that 80% of this insect pollination is carried out by bees. Both managed and wild pollinators are in decline worldwide. Numerous possible causes have been cited for this decline. Including disease, parasites, habitat loss, habitat fragmentation and intensive agrochemical usage, among others. Agrochemicals, particularly insecticides, have been critiqued intensively in relation to this decline. The neonicotinoids have faced particular criticism, leading to calls for the reduction of agricultural pesticide usage. However, it is imperative that we find a beneficial solution for all parties. Our agricultural economy relies heavily on pesticides to combat crop pests and diseases, in order to maintain the crop yields essential to meet growing food demand worldwide. Nevertheless, we cannot face the consequences of the loss of pollination services. This project aims to assess the sub-lethal effects of a range of insecticide classes on the mobility, navigation, memory and learning of the wild pollinator *Bombus terrestris*. The project utilises a novel bee behavioural assay using a thermal-visual arena. The arena facilitates the combined use of both appetitive and aversive conditioning to rapidly train individuals to return to a cool, 'safe' zone in an otherwise unappealing, hot arena. Bees' ability to learn and return to a safe zone is assessed using proprietary tracking software. These learnt behaviours will then be studied in response to field realistic levels of pesticide exposure. Further on in the project, the sub-lethal effects of pesticides on short and long term memory will be assessed by studying the direct effects on the bees' memory pathways, which are relatively well characterised at the biochemical and molecular level.

Keywords: Bumblebee, learning, memory, neonicotinoid, pesticide, behaviour

CO175

A COMPARATIVE TOXICOLOGICAL STUDY ON TOMATO RUSSET MITE (*ACULOPS LYCOPERSICI* MASSEE) AND ITS PREDATOR *AMBLYSEIUS SWIRSKII* ATHIAS-HENRIOT (ACARI: PHYTOSEIIDAE)

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The tomato russet mite [*Aculops lycopersici* (Massee) (Acari: Eriophyidae)] is common pest in tomato growing. In the early period, the mite damage looks like lack of plant nutrients in the lower leaves. Later, leaves become brown, rust symptoms are seem on stems and plant completely gets dry. In field, high population of the mite occurs generally from July to September when is beginning of tomato harvest period. In heated greenhouses, tomato production is carried out throughout a year and the harvesting continues periodically. In both cases, reducing pesticide residues, the best strategy is to increase effectiveness of natural enemies of this pest and to protect them. *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) is one of the predator of the pest. In IPM, determining the side effects on the predator mite for insecticide-acaricides recommended against *A. lycopersici* is critical point. In Turkey, the acaricide registered against tomato russet mite is highly toxic to the commercial strain of *A. swirskii*. The first aim of the study is to determine susceptibilities of *A. lycopersici* to 12 acaricide/acaricide-insecticide. The lethal doses (LD50, 90, 99) of abamectin, acequinocyl, bifenazate, bifenthrin, etoxazole, fenbutatin-oxide, hexythiazox, milbemectin, pyridaben, spiromesifen, spirodiclofen and tebufenpyrad for *A. lycopersici* were calculated by Probit analysis method using results of residual bioassay. In this study, the most toxic substances are abamectin, milbemectin and pyridaben for *A. lycopersici*. The second aim of the study is to test lethal toxicity of LD99 values calculated for *A. lycopersici* against larvae of a Turkish strain of *A. swirskii*. In addition, sublethal effects of the compounds on reproduction of female phytoseiid was determined by using the LD99 values. Based on the evaluation method suggested by IOBC, LD99 values of bifenthrin, etaxazole, fenbutation oxide, hexythiazox, tebufenpyrad, spiromesifen and spirodiclofen calculated for *A. lycopersici* were found moderate toxic against *A. swirskii*. Other acaricide-insecticide compounds were determined as slightly toxic to *A. swirskii*.

Keywords: *Aculops lycopersici*, *Amblyseius swirskii*, toxicology, side-effect, acaricide, insecticide, tomato

CO176

ARE ESSENTIAL OILS EFFICIENT AS BIOPESTICIDES AGAINST SAP-SUCKING PESTS? A BIOASSAY SCREENING AT DIFFERENT SCALES

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In the last decades, aromatic plant essential oils (EOs) have received growing attention as a low-risk alternative to pesticides in integrated pest management owing to their low mammalian toxicity and environmental impact. The toxicity of various Mediterranean EOs experimental formulations was evaluated to assess their insecticidal activity against two world-wide distributed sap-sucking pests, the silverleaf whitefly *Bemisia tabaci* (Gennadius) and the potato aphid *Macrosiphum euphorbiae* (Thomas). Two EOs ways of exposure - by contact or by fumigation - were tested in bioassays at leaf scale. All six oils showed dose-dependent insecticidal activity against both insects in contact experiments, but high mortality rates were always associated with severe phytotoxicity on tomato leaflets. This effect might have been accentuated as the polysorbate surfactant (Tween®) alone exhibited severe chlorosis in recommended concentration on tomato leaves, questioning its routine use in EOs bioactivity assessment.

Fumigation seems to be a good alternative to reduce phytotoxicity. This way of exposure allowed Rosemary and Mugwort oils to be highly effective against *B. tabaci*, as well as green anise and fennel oils against *M. euphorbiae*, all inducing more than 90% of mortality due to a suspected neurotoxic effect at non-phytotoxic doses. This effect was partly found again at plant scale but also at greenhouse scale. In order to identify the blend of active compounds, the volatile constituents of EOs were analyzed by a combination of GC-FID/MS. This study provides a new potential resource to develop more eco-friendly means of sap-sucking pest control in greenhouse crops through essential oil vapour-phase exposure.

Keywords: Entomotoxicity, phytotoxicity, ecotoxicology *Bemisia tabaci*, *Macrosiphum euphorbiae*, tomato crop, biocontrol, IPM strategies

CO177

NEONICOTINOID INSECTICIDES AND BEE POLLINATORS

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Neonicotinoids are an important class of insecticides which are applied worldwide in a great variety of crops. Due to their systemicity they can be used as foliar sprays as well as systemic seed treatment. In the last years, the safety of neonicotinoid products to bees has been controversially debated, which has eventually led to regulatory restrictions in the European Union. We provide here an overview of the available data on the bee safety of neonicotinoids, and on the current state of the debate. Part of the neonicotinoid compounds is, as most insecticides, intrinsically toxic to bees, whereas others have a low inherent toxicity. For the intrinsically toxic compounds, mitigation measures are in place to minimize exposure. In the case of seed treatment, bees may be exposed to trace level systemic residues in nectar and pollen. Different potential exposure routes for bees are discussed. For the evaluation of most of the products, higher-tier studies were conducted under realistic field conditions. In most of these studies representative for practical conditions, no adverse effects were observed, whereas under artificial worst-case conditions in the laboratory negative effects were frequently seen. Concerns were brought up about sublethal effects which might affect behavior, cognition, or orientation of bees, without directly killing them. However, a concrete adverse impact of potential sublethal effects to bee colonies or populations under realistic field conditions has not been demonstrated. In 2013, the European Commission restricted the use of three neonicotinoids in bee-attractive crops. This step remains controversial, since it was based on a new proposed evaluation system which has to date not been officially implemented, and which is still disputed in the scientific and the regulatory community. Five years after the restrictions have been stipulated, available data show that they have not led to an improvement of bee health in Europe.

Keywords: bees, pollinators, neonicotinoids, insecticides

CO178

SUBLETHAL SIDE EFFECTS OF SYSTEMIC INSECTICIDES ON *HARMONIA AXYRIDIS*

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Systemic neurotoxic insecticides are widely used for controlling aphid pest populations in agricultural crops. They can affect the activity of beneficial organisms, such as coccinellid predators. Typically, the endpoints of studies on non-target risk assessments are the evaluation of survival and/or reproductive traits. However, behavioral responses linked with ecological control services (*e.g.* voracity), could be crucial when assessing the compatibility of pesticide with biocontrol agents. We exposed for 3 days adult females of *Harmonia axyridis* (harlequin ladybird) to plants and aphids sprayed with two neonicotinoids (imidacloprid and thiamethoxam) and a sulfoxamine (sulfoxaflor) insecticide. For this, we applied the chemicals at their Lethal Concentrations 50, 20 and 1, previously estimated for the target organism, the polyphagous cotton aphid, *Aphis gossypii*. The survival and the functional response to the prey density of the predators after feeding on treated prey and being exposed to pesticide residues were thus assessed. The results showed that the survival and the voracity differed among the tested chemicals and concentrations. Although only thiamethoxam at LC50 caused a significantly higher mortality, insecticides did not show relevant acute toxicity. In most of the pesticide-concentration combinations, the predators showed a density independent functional response, while an inverse density dependence of the prey consumption rate was observed for coccinellids exposed to sulfoxaflor and thiamethoxam at their lowest concentration. The estimated parameters, *i.e.* the attack rate and the prey handling time, were negatively affected at higher concentrations by both imidacloprid and sulfoxaflor. Our findings stress the importance of carefully evaluating the potential behavioral side effects of insecticides on natural enemies, even at very low concentrations.

Keywords: Biocontrol, Ecotoxicology, Neonicotinoid, Risk assessment

CO179

EFFECTS OF PESTICIDE COCKTAILS ON BEES

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Bees in agricultural and urban environments are often exposed to combinations of pesticides. Multi-exposure may occur when bees forage on crops treated with various pesticides, either applied sequentially or in tank mixes, as well as when bees forage on various flowers contaminated with different compounds. As a result, studies analyzing chemical residues in bees and bee matrices such as honey, pollen and wax, often find several compounds (typically at low concentrations). These multi-residue field scenarios are not accounted for in regulatory risk assessment schemes, which are based on tests of single products. This is an important shortcoming because pesticide mixtures may have not only additive but also synergistic effects. Yet, information on the ecotoxicology of pesticide mixtures is mostly lacking. We have found synergistic lethal effect between a common neonicotinoid and an EBI fungicide at sublethal concentrations in three bee species (*Apis mellifera*, *Bombus terrestris* and *Osmia bicornis*). In *O. bicornis* we have also found synergistic effects on ovary development. Our results underscore the need to consider realistic multi-residue exposure if we are to understand the effects of pesticide use in realistic field situations. There are more than 20,000 bee species worldwide, encompassing a huge diversity of life history traits. Differences among species entail different routes and levels of exposure, as well as differences in sensitivity to pesticides. Our results also emphasize the need to include additional species besides the European honey bee, *A. mellifera*, in regulatory risk assessment.

Keywords: Ecotoxicology, synergism, *Apis mellifera*, *Bombus terrestris*, *Osmia bicornis*

CO180

DETRIMENTAL SYNERGISTIC EFFECTS OF THE PESTICIDES ON THE SURVIVAL OF PARASITOIDS

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In conventional agriculture, the abundance of pests, pathogens, and weeds in crop fields are controlled by synthetic insecticides, fungicides, and herbicides. Consequently, the use of pesticides has been a large contributor to decreases in beneficial insect abundance and diversity. For example, research has indicated lethal and sublethal effects of pesticides on numerous parasitoid wasp species of economic importance. Here we examine the effects of relevant pesticide binary mixtures on adult parasitoid wasp mortality and functional motor skills (FMS). Glass plates were treated in a spray tower, and subsequently used to construct cages where both direct mortality and loss of FMS were recorded 2, 4, 6, 8, and 24 hours post-exposure to dry treatment residues. In the first part of the study, the pesticidal active ingredients thiacloprid (neonicotinoid insecticide), tau-fluvalinate (pyrethroid insecticide), tebuconazole (ergosterol-biosynthesis-inhibiting fungicide), and glyphosate (herbicide) were tested on parasitoid wasps (*Aphelinus abdominalis*, *Diglyphus isaea*, and *Aphidius matricariae*), using commercial formulations, and examined alone and in binary mixtures. In the second part of the study, selected binary mixtures that showed significant synergistic effects were examined further on *A. abdominalis* mortality and FMS by manipulating the amount of fungicide in the mixtures, in order to examine the effects of ratio manipulation on synergy dynamics.

Keywords: Neonicotinoid, pyrethroid, fungicide, glyphosate, mortality, functional motor skills

Session 6.
Ecology, Biodiversity and Conservation

Arthropod-Mediated Ecosystem Services in Agriculture

CO181

COMPLEXITY OF FACTORS THAT AFFECTS POLLINATORS – FARMING INTENSITY, CLIMATE AND BELOW GROUND PROCESSES

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Pollination is one of the most important ecosystem services, and it is in the focus of both science and policy. There are, however, many drivers that may affect pollinators and pollination success. We provide examples on the effects of three major drivers on pollination. Farming intensity, *e.g.* the increased use of insecticides and other practices of conventional farming lead to the decline of pollinators, mainly wild bees, resulting in a pollination deficit. Still, there are low intensity farmlands in Eastern Europe, which have diverse pollinator communities, demonstrating a baseline for a pollinator-rich farmland. Diverse pollinator community, in turn, is needed to provide insurance against *e.g.* climate change. Warming of spring temperature is expected, which may cause a phenological mismatch between wild plant and crop flowering, and diversity or peak of pollinator abundances, simply due to different speed of adaptation to environmental change. In an experiment we showed that pollinator diversity can ensure pollination for both delayed and advanced flowering of apple trees, as different pollinator group was dominant at different climate-treatments. Finally, pollination is affected not only by farm management and other environment factors, but also by hidden indirect ecological drivers such as below ground processes. We found in a mesocosm experiment that pollinator visitation of the marjoram was highest, if both earthworms and arbuscular mycorrhiza were added to sterile soil, indicating that fertile soil indirectly contributes to higher attractiveness of flowers for pollinators. Our studies show that sustainable farming needs diverse pollinator assemblages under climate change, and also appropriate management of above and below ground environment. In addition, diverse pollinators may contribute to ecological intensification which can serve as a solution through the enhancement ecosystem services delivery to increase crop yield instead of chemical inputs and intensive farming practices.

Keywords: Wild bees, East Europe, phenological shift, below ground effects

CO182

BIODIVERSITY AND ASSOCIATED ECOSYSTEM SERVICES IN SMALL VS LARGE SCALE AGRICULTURE

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Agricultural intensification drives biodiversity loss and increases farmers' profit, but the role of legacy effects for ecological-economic trade-offs are largely unknown. In Europe of the 1950ies, the Eastern communist bloc switched to large-scale farming due to forced collectivization of small farms, while the West kept small-scale private farming. We found that large-scale agriculture in East Germany reduced biodiversity, which has been maintained in West Germany due to >70% field edges, in favour of 50% higher profit per farmland area due to marketing advantages despite similar yield levels. In both regions, switching from conventional to organic farming increased biodiversity, halved yield levels, but doubled farmer's profit due to effective marketing. However, in general these biodiversity effects did not translate into more efficient biocontrol services measured as aphid parasitism, natural enemy/aphid ratio, predation on aphid cards and weed seed predation by beetles. Only the vegetation dwelling predator-aphid ratio was higher in West *vs* East (mostly due to differences in conventional fields). Nevertheless the absence of clear and strong effects of region, management or edge vicinity on these biocontrol measures implies that either pest infestation did not reach the economical harmful threshold or the relative high amount of natural enemies and/or pesticide application could keep them under control. In conclusion, agri-environmental schemes should acknowledge neglected benefits of small-scale agriculture for biodiversity and its potential services.

Keywords: Arthropods, biocontrol, edge effect, field edges, landscape heterogeneity, organic farming, pest control

CO183

MULTILEVEL PLANT DIVERSITY FOR ENHANCED CONSERVATION BIOLOGICAL CONTROL AND CROP SYSTEM RESILIENCE

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To feed the world's growing population, agriculture has to become more productive but also to ensure social, environmental and economic sustainability. Special attention is paid to the reduction of the use of inputs, especially pesticides in cropping systems. In conservation biological control, many initiatives aim at increasing interspecific plant diversity within fields or in their vicinity, in order to reduce pest infestation and to favour the establishment and survival of beneficial arthropods. Genetically diversified crop, *e.g.* mixtures of different cereal varieties, has been shown to support lower aphid populations than one crop variety alone. Moreover, increasing plant diversity through species association, *e.g.* cereal and legume mixed cropping, has also been shown to improve productivity and pest control. Cereals and legumes intercropping can improve utilization of available soil resources and therefore result in an increase of yield performance and stability compared to monoculture. Such agroecological practice seems also to reduce aphid populations and to increase densities of ladybirds, hoverflies and parasitoids compared to monoculture. In South-East of France, on-farm experiments have been conducted during 2 years to investigate the synergistic effects of increasing plant functional diversity at different levels (crop varieties and species) on crop performance and on interactions between insects and crops with a particular focus on potential for biological control. Cropping system investigated were organic wheat systems with sole wheat crop, the combination of different wheat varieties, and wheat-clover intercropping. Arthropods monitoring focused on aphid pest and some natural enemies (ladybirds, syrphids, carabids, lacewings, spiders) through visual observation, pitfall traps, sentinel prey and artificial caterpillars. This talk will present the concept of stacking and some results from field research.

Keywords: Crop associations, variety mixture, pest, beneficial insects, sentinel prey

CO184

ARE PARASITOID WASPS LIMITED BY SUGAR SOURCES IN AGRICULTURAL AREAS? POMEGRANATE ORCHARDS AS A CASE STUDY

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Sugar from nectar or from honeydew can prolong parasitoids' lifespan, enhance their fecundity and foraging ability, and thereby increase their pest suppression efficiency. Sugar sources within crop monocultures are considered to be limiting for parasitoids. Nevertheless, only few studies have measured the sugar feeding of parasitoid assemblages in agricultural areas or in surrounding non-crop habitats. We used cold anthrone tests to compare the frequency of sugar feeding in parasitoid communities, inside pomegranate orchards and in adjacent natural areas, over two consecutive years. Overall, 40% of the 1610 sampled individuals belonging to 135 species scored positive for sugar. Sugar-feeding frequency was lower within the orchards than in the neighboring natural areas. The proportion of sugar-fed wasps increased with herbaceous vegetation cover, both within and outside the orchards, suggesting that herbs are a sugar-rich habitat. Parasitoids sampled from trees and from herbs within the orchards had similar frequencies of sugar feeding, despite differences in wasp species composition. Our results probably overestimate sugar-feeding frequencies in the field because sugar-fed individuals have higher longevity and hence are more likely to be sampled. We propose a simple model to approximate this over-sampling bias and apply it to *Encarsia inaron* (Aphelinidae), one of the most abundant parasitoids in the samples. We conclude that sugar availability potentially limits parasitoid fitness in this agro-ecosystem. This may be due to the low density of plants in natural areas during the Mediterranean summer, and to herbicide applications within the orchards that further suppress non-crop herbs.

Keywords: Sugar feeding, cold anthrone test, nectar, honeydew, *Encarsia inaron*

CO185

THE SPECIES-HABITAT NETWORK: A NOVEL APPROACH TO THE STUDY OF HABITAT USE

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A deep understanding of how species use habitat resources scattered through the landscape is pivotal for biodiversity management. Traditional approaches to this subject have mainly been based on the dichotomy between patches of a focus habitat type and the surrounding landscape matrix; this viewpoint, however, fails to fully capture the complexity of real-world landscape mosaics. We propose a novel solution to the problem by modelling bipartite networks between multiple habitats and species, in analogy with the mutualistic networks used to model plant-pollinator interactions. The versatility, visualization power and easy interpretation of the species-habitat network enable its application to a wide range of real-world conservation issues, including the effects of land use intensity on biodiversity. In this study, we selected 15 landscapes, representing gradients of semi-natural habitat amount and configuration (which are proxies for land use intensity), and placed pitfall traps in 20 different semi-natural and agricultural habitat patches in each landscape. The main focus organisms were ground beetles (Coleoptera: Carabidae), as they are very abundant, relatively easy to identify and important both as bioindicators and biocontrol agents. Each landscape was modelled as a bipartite network between the ground beetle species and the sampled habitat patches. Network metrics suggest a significant correlation between habitat specialization of ground beetles and semi-natural habitat features, at both species and network level. Implications of these findings for biodiversity conservation and ecosystem services management are discussed.

Keywords: Bipartite networks, Landscape ecology, Carabidae, Ecosystem services, Landscape management

CO186

DESIGNING AGRICULTURAL LANDSCAPES TO ENHANCE ARTHROPOD-MEDIATED ECOSYSTEM SERVICES

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Sustainable and resilient agricultural systems are needed to feed and fuel a growing human population. However, the current model of agricultural intensification which produces high yields has also resulted in a loss of biodiversity, ecological function, and critical ecosystem services in agricultural landscapes. A key consequence of agricultural intensification is landscape simplification, where once heterogeneous landscapes contain increasingly fewer crop and non-crop habitats. Landscape simplification exacerbates biodiversity losses which leads to reductions in ecosystem services on which agriculture depends. In recent decades, considerable research has focused on mitigating these negative impacts, primarily via management of habitats to promote biodiversity and enhance services at the local scale. While it is well known that local and landscape factors interact, modifying overall landscape structure is seldom considered due to logistical constraints. I propose that the loss of ecosystem services due to landscape simplification can only be addressed by a concerted effort to fundamentally redesign agricultural landscapes. Designing agricultural landscapes will require that scientists work with stakeholders to determine the mix of desired ecosystem services, evaluate current landscape structure in light of those goals, and implement targeted modifications to achieve them.

Keywords: Biodiversity, Ecosystem Functions, Ecosystem Services, Landscape Simplification

CO187

POTENTIAL INTERACTIONS BETWEEN ECOSYSTEM SERVICES DELIVERED BY INSECTS

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Yield formation is sustained and regulated by bundles of ecosystem services delivered by above- and belowground biodiversity. Ignoring potential interactions occurring between different ecosystem services may lead to unwanted tradeoffs and synergies, resulting in unexpected outcomes when turned into management practices. Although insect pollination is an important service to agriculture that has recently received great attention, it has been usually studied in isolation. Here, we compiled and reviewed published empirical studies on the contribution of insect pollination to crop yield under different ecological contexts. We found that interactions between pollination and other factors influencing yield were prevalent across several crops and, when present, they most frequently displayed positive-synergistic relationships. In particular, soil properties and crop pests affect pollination benefits by altering the amount of resources a plant can allocate to reproduction, independent of the amount of pollen provided. Management practices

that aim to augment insect pollination should include not only measures to enhance pollinator abundance and diversity but also measures to improve soil fertility and pest regulation. Expanding our knowledge about service interactions will improve our ability to define sustainable management strategies both at the local and regional scale.

Keywords: Bees, Biocontrol, Crop yield, Landscape, Pest regulation, Pollination, Soil services

CO188

SEASONAL DIFFERENCES IN THE EFFECTS OF COMMERCIAL BUMBLEBEES ON FRUIT QUALITY IN STRAWBERRY CROPS

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Managed colonies of commercially-reared honey-bees and bumblebees significantly contribute to global food production by providing pollination services to crops. Commercial bumblebees are increasingly used to pollinate soft-fruit crops, such as strawberry, an economically important crop globally. Despite the use of commercial bumblebees in strawberry, there is little quantitative evidence that they provide a benefit to farmers. Given the negative impacts that the importation of commercial colonies can have on wild bee populations, it is vital that the benefits of commercial bumblebees are quantified, so management decisions can be made that provide maximum benefit to farmers and wild bees. In this study, commercial bumblebee (*Bombus terrestris audax*) colonies were placed into June-bearer (flowering March-April) and everbearer (flowering May-June) strawberry polytunnels on a soft-fruit farm in the south-east of England, and opened and closed at weekly intervals. The flower-visiting assemblage inside polytunnels was quantified, and fruit was quality assessed. In the June-bearer crop, the presence of commercial bumblebees increased the amount of high commercial-grade fruit by 17.5%. This increase in quality is estimated to be worth approximately £16 million to the UK strawberry market alone. In contrast, no benefit of commercial bees on pollination or fruit quality was observed in the everbearer crop. The increase in quality of fruit in the June-bearer crop may be driven by higher *B. terrestris* flower visitation rates. The number of flower visits by wild pollinators was not a significant predictor of strawberry quality, thus their role in this system remains unclear. The results presented here suggest that commercial bumblebees can greatly increase the quality and value of strawberry crops. However, interactions between commercial bees, farm management practices and environmental factors may reduce their efficacy at certain times of year. Thus, careful consideration of such factors should be given before using commercial bumblebees on a crop.

Keywords: Pollination, ecosystem service, commercial *Bombus terrestris*, fruit quality, Fragaria, Nitidulidae, crop

CO189

MOLECULAR ASSESSMENT OF INVERTEBRATE FOOD WEBS: CURRENT APPLICATIONS AND FUTURE OPPORTUNITIES

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DNA-based methods have significantly widened our ability to assess trophic interactions under natural conditions. With conventional methods, these were before difficult to impossible to study. The technical advancement within the last decade enables us nowadays to empirically examine complex feeding networks, including plant-herbivore, predator-prey and host-parasitoid interactions. This talk will exemplify how molecular techniques have been employed to unravel food web interactions and how these trophic networks respond to environmental changes across a range of systems – from arable land to glacier forelands. In addition to the exciting opportunities DNA-based approaches offer, we will also highlight current challenges and future opportunities for assessing multiplex feeding networks in invertebrate systems.

Keywords: Molecular trophic ecology, multiplex food webs, real world feeding networks

CO190

PLANT DIVERSITY EFFECTS ON APHID BIOCONTROL THROUGH TOP-DOWN SELECTION OF APHID MICROBIAL SYMBIONTS

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Plant diversity affects ecosystem functioning through its effect on biotic interactions. In experimental grasslands, increasing plant species richness is known to increase the diversity of associated herbivores and their predators. These interactions can also involve endosymbionts that reside within a plant or animal host. In plant-feeding aphids, secondary bacterial symbionts can have strong fitness effects on the host, *e.g.* resistance to natural enemies or fungal pathogens. In this talk we explore how plant diversity may act on the aphid symbiont community through affecting aphid natural enemies, and discuss consequences for aphid control in agro-ecosystems. In the Jena biodiversity Experiment we examined the symbiont community in three species of aphids. We found that at the aphid population level, increasing plant species richness increased the diversity of the aphid symbiont community, whereas at the individual aphid level the opposite was found. We then set up a pot experiment where barley was grown without other plants, with one of three wildflower species, or with all three species. Population sizes of the two main aphid species that colonized barley plants were reduced in the presence of wildflowers, with a stronger reduction when all wildflowers were present. Natural enemy diversity was highest in the 3-species treatment, and specialist aphid parasitoids were most abundant in the 3-species treatment. There was a positive association between the proportion of aphids hosting the symbiont Regiella and natural enemy diversity, at both the individual aphid and local population level. Our work extends documented effects of plant diversity beyond visible biotic interactions to changes in endosymbiont communities. The results are consistent with the hypothesis that flowering plant diversity increases the diversity of symbionts within local aphid populations, through increased selection pressures related to natural enemy diversity, and this reduces aphid population size hence increasing aphid biocontrol.

Keywords: Aphid, symbiont, biodiversity

Conservation of Saproxylic Insects

CO191

CONSERVATION OF SAPROXYLIC INSECTS

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Saproxylic insects have been recognized for a long time as pests producing damage on trees. The Habitats Directive brought many insects into focus of nature conservation and saproxylics became a key organism group in conservation planning. Strong efforts have been put into methodological improvements for the monitoring of species and communities. A better knowledge on species distributions and their dynamics in space and time is required to develop effective conservation strategies. I will summarize more than 20 years of studies on saproxylic insects and will particularly discuss some current hot topics in saproxylic insect conservation: 1) Integrative *vs* segregative forest conservation strategies, 2) Dispersal ability and habitat connectivity, 3) the role of primeval forest relict species for conservation planning.

Keywords: forest Management, dispersal, Habitat connectivity, saproxylic beetles, primeval relicts, conservation strategies

CO192

DIVERSITY OF SAP BEETLE FAUNA OF INDIA: STATE OF OUR KNOWLEDGE (COLEOPTERA: NITIDULIDAE)

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The global species richness and diversity estimates of the beetles have been a debatable matter of the experts. These are far more pertinent for the minute beetles due to their cryptic habitats, as well as insufficient explorations and taxonomic analysis. The ‘sap beetles’ or the Nitidulidae are a distinct representative of these minute beetles, which are primarily saproxylic. The community of these saproxylic beetles is shaped by different assemblages of plant and animal species linked by their microhabitats. They exhibit diverse forms and sizes [0.9 to 15 mm long], varied structural features and functional attributes. They are also economically important and include pests of stored grains and fruits, pollinators of flowers; some are detrimental to honey bee colonies and useful in forensic science. Despite their wide habitat range and important ecosystem services, these beetles did not receive due attention of the taxonomists of India in the past. Standing in a transition zone between the Palaearctic and Oriental Realms, Indian landmass exhibits multitudes of ecological conditions and forests in different parts of the country. It has contributed to a diverse sap beetle fauna in this land. The fauna however, was not well explored and taxonomically worked out. Hitherto, about 177 species belonging to 45 genera (out of 4500 species and 350 genera globally) were recorded from India. A recent study by us in North-East India only revealed 60 species belonging to 14 genera including 13 new species. It indicated that the present taxonomic knowledge of the sap beetles would increase substantially with thorough field exploration and fair taxonomic analysis of the faunal material. These beetles are also one of the most threatened insect groups as the forests and vegetation are facing increasing man-made pressures. Better taxonomic knowledge and their conservation approaches are therefore necessary for the welfare of humanity.

Keywords: Beetle, Saproxylic, Oriental, Taxonomy, Habitat, Conservation

CO193

STATUS OF *ANEURETUS SMONI* EMERY (HYMENOPTERA, FORMICIDAE) FROM REPEATED SAMPLING AND A PRELIMINARY ANT INVENTORY OF LENAGALA FOREST RESERVE, SRI LANKA

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The Sri Lankan Relict Ant, *Aneuretus simoni* Emery, was recently reported from several forests in the wet and intermediate zones of Sri Lanka including Lenagala Forest Reserve that lie in Kegalle District of the wet zone. The survey on the ant community of Lenagala Forest Reserve was extended by visiting the forest four times, September and November in 2016 and February and August in 2017, to prepare a preliminary ant inventory and to estimate the nest density of *A. simoni* for the determination of its status using quadrat method, frequency of nest occurrence (FNO%) and the frequency of worker occurrence (FWO %) using the pitfall traps. Twenty, 1x1m^2 quadrats were laid covering each of the two, 100m^2 plots marked at each 255 m and 280 m elevation. Three worker ants from each ant nest within each quadrat were collected. Also, 100, honey-baited pitfall traps at 4 m distance were fixed on the ground along eight transects marked throughout each sampling region and collected after 6 hours. In addition, soil sifting was conducted at 1 m distance along the 8 transects from 23-25 June only in 2017. All collected ants were preserved in 80% ethanol and labelled appropriately. Air and soil temperature, depth of litter, soil moisture% and soil organic matter% at three representative places in each plot were recorded to characterize the habitat of *A. simoni*. Fifty one species in 32 genera of 6 subfamilies, Aneuretinae, Dolichoderinae, Dorylinae, Formicinae, Myrmicinae and Ponerinae, were listed by pooling the ant taxa recorded from the four visits and can be considered the preliminary ant inventory of the forest. Species richness values, 31, 35, 30 and 37, were recorded by the quadrat method and pitfall trapping conducted on each occasion. Soil sifting alone resulted 30 species. *Aneuretus simoni* was among the 12 resident species while its nest density ranged from 0.08-0.14m-2, FNO varied from 8%-14% and FWO ranged from 10% - 18% on the four occasions. Ranked mean nest density showed that *A. simoni* had 8th, 6th, 7th and 5th rank, respectively and it is a permanent resident of the ant community in Lenagala Forest Reserve in Kegalle District.

Keywords: Sri Lankan Relict Ant, ant diversity, Forest reserve, ant sampling methods

CO194

CONSERVATION STATUS OF SAPROXYLIC INSECTS IN RELATION TO THE SPATIOTEMPORAL DYNAMICS OF THEIR OVIPOSITION SUBSTRATES: LESSONS FROM PHEROMONE MONITORING OF AN EXPANDING ARRAY OF MODEL SPECIES

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Reliable data regarding distribution and population trends could facilitate evidence-based conservation efforts for many saproxylic insects, which can be very difficult to monitor with traditional methods. We have identified and applied insect sex and aggregation pheromones to monitor rare and threatened saproxylic insects in a North European context, with unprecedented accuracy at local and regional scales. For some species, our pheromone monitoring efforts constitute the first systematic and reliable records of their true distributions, and also provide comparable and objective estimates of relative abundance. While the number of pheromones identified for conservation purposes remains limited, the saproxylic species covered so far nevertheless represent a diverse selection of ecological niches. This is partly represented by a variety of host trees, but even more so in terms of different dynamics with regards to generation and persistence of deadwood substrates. Model species that we have studied in a landscape context comprise e.g longhorn beetles that exploit early-successional and ephemeral resources such as newly dead branches and twigs, to later successional larger branches and trunks. Other insects, such as the longhorn tanner beetles *Prionus coriarius* and *Tragosoma depsarium*, but also tineid moths, exploit substrates characteristic for later successional stages with higher persistence. Yet other groups of insects, such as the hermit beetle *Osmoderma eremita* and the rust red click beetle *Elater ferrugineus*, depend on centennial substrates like tree hollows. Even limited sets of saproxylic indicator species, monitored with high precision, thus provide the possibility to capture a broad range of general processes reflecting different spatiotemporal dynamics in the availability of resources, just by comparing their abundance in different types of habitats. These systems allow for testing hypotheses linking conservation status to saproxylic resource niches with specific spatiotempral dynamics, and provide robust models for focusing habitat management regimes to the needs of specific insect groups.

Keywords: Saproxylic, Spatiotemporal dynamics, Pheromone monitoring, Conservation biology, Landscape ecology

CO195

THE CITIZEN SCIENCE APPROACH IMPLEMENTED IN THE LIFE PROJECT “MONITORING INSECTS WITH PUBLIC PARTICIPATION” FOR THE CONSERVATION OF SAPROXYLIC BEETLES

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The LIFE Project “Monitoring Insects with Public Participation” (MIPP, LIFE11 NAT/IT/000252) is the first LIFE project which gathers records of saproxylic beetles listed in the “Habitats” Directive by means of volunteers. The dataset collected so far, by more than 800 citizens in four years of observations (2014-2017), amounts to over 3000 records (of which 75% were confirmed by specialists) that were delivered thorough website or smartphone app. This dataset could be used to address questions related to several aspects on biology, ecology, morphology, systematics and conservation of species. Some case studies will be presented. We compared the data of the species with the highest number of records (Lucanus cervus, Morimus asper/funereus and *Rosalia alpina*) obtained by the project with the data of the official Italian species inventory. The results of the analysis showed a significant increase in the distribution range of the considered species thanks to the “MIPP citizen-scientists”. Moreover, we analyzed the altitudinal distribution and phenology of the species.

Keywords: Citizen Science, Saproxylic, Habitats Directive

CO196

QUANTIFYING THE SHORT-TERM EFFECTS OF LOGGING IN OAK DOMINATED FORESTS ON THREATENED LONGHORN BEETLES (CERAMBYCIDAE) WITH A PHEROMONE-BASED TRAPPING SYSTEM

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Saproxylic longhorn beetles include a large number of threatened species, indicator species and flagship species for insect conservation. However, many species are problematic to study quantitatively as they are dependent on ephemeral substrates such as fresh, recently dead wood, a habitat which is difficult to sample with conventional survey methods. Recently, we have identified the aggregation-sex pheromones of several longhorn beetles dependent on fresh, dead oak wood in order to construct an efficient tool for detection, quantification and monitoring of their local abundance. Using a pheromone-based trapping procedure, we conducted a two-year study measuring beetle abundance of seven species at 52 oak dominated forest stands and woodlands in Sweden. The sites were categorized into four types; treatment stands, controls, key biotopes and hotspots. Treatment stands were forests which had undergone recent logging activity with comparatively large amounts of fresh, dead oak wood (primarily twigs and branches). Controls were similar, but untreated, stands which had much smaller amounts of dead oak wood. The key biotopes are small set aside forests specifically intended to benefit biodiversity while hotspots are sites well known for their importance to threatened saproxylic beetles associated with oak. In general, treatment stands had a higher average number of beetles compared to the controls, but the strength of the effect varied depending on species. Surprisingly, key biotopes and hotspots did not perform better than the controls and had fewer beetles than the treatments. We conclude that thinning or logging in oak forests can benefit many species in this group of longhorn beetles, provided that some of the wood material is left on site, but ecologically demanding species, *e.g.* Poecilium pusillum and Plagionotus detritus, will require more specific conservation actions. In addition, pheromone-based monitoring appears to be a promising tool to study this group of elusive insects under field conditions.

Keywords: Monitoring, nature management, conservation, Red List, pheromone, cerambycid

CO197

Abstract moved.

CO198

SAMPLING OF RARE AND ENDANGERED SAPROXYLIC BEETLES FOR MONITORING AND CONSERVATION: NEW PERSPECTIVES

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The EU Habitats Directive directed that monitoring of species from the Annex II list should be established in Europe. Among listed species there are many poorly known and rare saproxylic beetles. In the past for most of those only anecdotal or occasional records existed, which were in many countries the only data used for designation of Natura 2000 sites. In recent years, methodological studies on field sampling approaches for these species were greatly intensified in order to reveal species distribution and abundance and to establish monitoring schemes for assessing long-term population trends. This contribution will summarize recent achievements of the development of survey protocols as well as compare results of different approaches. There are three major groups of survey methods currently used for monitoring and survey of endangered saproxylic beetles: (1) citizen science method (collecting of occasional species records; presence data only), (2) conventional survey methods (systematic observational surveys or different kind of traps developed from observational and collecting experiences; presence/absence and abundance data), and (3) communication lure methods (advanced methods based on the species communication system, in saproxylic beetles mainly chemical communication with semiochemicals; presence/absence and abundance data). As a case study, two species will be used, the Hermit Beetle (*Osmoderma eremita*) and Alpine Longhorn (*Rosalia alpina*), in which recent studies on chemical communication enabled development of advanced survey methods using pheromones that were tested on large-scale in the field. The efficiency of new methods as well as new insights into species biology due to the use of chemical lures will be presented.

Keywords: Survey method, method evaluation, *Osmoderma eremita*, *Rosalia alpina*, monitoring

CO199

IDENTIFICATION OF A MALE-PRODUCED AGGREGATION PHEROMONE FOR ENDANGERED BEETLE ROSALIA ALPINA AND AN ATTRACTANT FOR THE PREDATORY CLICK BEETLES

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In the past decade, extensive progress has been made in identifying pheromones of endangered insect species. The Alpine longicorn (*Rosalia alpina*, Cerambycidae) represents one of the indicator species of the saproxylic biodiversity in Europe. Recent studies have documented great declines in its populations, as a result of habitat loss. In this study, volatiles were collected from mature male and female *R. alpina* and a compound produced specifically by mature males was identified as a novel alkylated pyrone structure. In field tests at three locations in Slovenia, multiple-funnel traps baited with the synthesized pyrone captured both sexes of *R. alpina*, indicating that the compound functions as an aggregation pheromone. Results demonstrated that pheromone-baited traps could provide a useful tool for sampling Alpine longicorn and facilitating the development of conservation strategies for this endangered species. In addition, the study showed that *R. alpina* aggregation pheromone had a significant trapping effect also on click beetles, indicating that the compound could also function as a kairomone. We have investigated the possible roles of this pheromone in interspecific interactions, in particular weather click beetles use it for prey detection, as an indirect cue for mate location and/or as a habitat signal.

Keywords: *Rosalia alpina*, pheromone-based monitoring, interspecific interactions

Insect Species Traits and Their Use in Habitat Management and Conservation

CO200

WHAT TRAITS CAN TELL US ABOUT GROUND BEETLE ECOLOGY AND CONSERVATION

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Traits are becoming increasingly popular in organismic biology, especially for ecological and nature conservation biological analyses. This is due to the fact that life history traits, but also distributional traits suggest a mechanistic explanation for many phenomena ranging from community composition and trophic interaction networks to the extinction of species. As a model group we use carabids, a relatively well studied beetle family that occurs in almost all terrestrial habitats with numerous species. In addition, traits of ground beetles have been used for many years. A dynamic data base exists which comprise a large set of life history and distributional traits for many European species. We provide an overview of trait analyses and their results and focus on the recent studies dealing with long-term investigations. The results show that many studies deepen our understanding of adaptation processes to changing environmental conditions. Other studies reveal the traits and their characteristics leading to an increasing risk of extinction. The need for further studies is obvious, especially on the importance of phylogeny for the traits. Moreover, a critical examination of traits and their application also shows which pitfall traps exist for entomologists, not only carabidologists.

Keywords: Carabidae, life history traits, distributional traits, ground beetles, conservation biology, climate change

CO201

BUTTERFLY TRAITS AND VULNERABILITY AT COMMUNITY AND SPECIES LEVEL IN CHANGING TEMPERATE ENVIRONMENTS

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Trait-based studies allow to gain a more mechanistic understanding of environmental impacts on ecosystems, and generate less context dependent results than those only based on taxonomic inventories. Butterflies are better suited to this kind of studies than most other invertebrate taxa because of the considerable amount of information now available on their ecology, morphology, behaviour and life-history traits. Many papers also concur that butterflies tend to respond to continued environmental change, such as climate change, forest encroachment, or agricultural intensification, more promptly than other taxa. The butterfly communities most severely affected by environmental change are basically those including species characterised by: 1) restricted range, 2) low mobility, 3) larval monophagy and 4) short flight periods (monovoltine), 5) altitudinal specialist. At species level, those diapausing as eggs or larvae, rather than as pupae or adults, are variously affected by variation in similar environmental traits, depending on the area. Particularly in disturbed environments, high species diversity does not always mean high functional diversity. Communities occurring in strongly anthropic areas tend to include species characterised by: a) High mobility, b) broad geographic range, c) low population density, d) multivoltine development. Especially in mountain ecosystems, community level variation in species composition and trait representation can be observed even in the short period. This is partially because high altitude communities are strongly subject to environmental filtering, are usually species-poor, and do not represent random subsamples of lowland communities. Butterfly traits can be investigated also at other levels, such as evolutionary or biogeographical, as a tool to explain species coexistence and community assembly rules, at various spatial and temporal scales.

Keywords: Range, mobility, monophagy, phenology, altitudinal specialist

CO202

THE CONSERVATION OF TRAITS IN GENERALIST PREDATOR COMMUNITIES: WHEN IS A TRAIT FUNCTIONAL?

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A functional trait may be defined as morphological, physiological or life-history characteristic that influences individual performance or fitness. More precisely, traits can be defined as effect traits if they relate to levels of functions provided by organisms or as response traits if they relate to the sensitivity of organisms to environmental change. Anthropogenic disturbances are known to affect arthropod communities in agricultural landscapes, with severe consequences for the provision of regulating ecosystem services such as biological control. Generalist predators are among the economically most important functional arthropod groups in agricultural landscapes, as they significantly contribute to animal and plant pest control. Yet, very little is known about response traits that characterize effects of environmental change on these communities at different spatial scales. Even less is known about effect traits that lead to changes in the provision of biological control services. In this overview, the accumulating evidence for strong response trait patterns in generalist predator communities is highlighted at landscape, ecosystem and within field spatial scales. Based on these patterns, environmental change can alter the functional role of generalist predators in local food webs at the respective spatial scale. Evidence for these changes is exemplified by presenting results on indirect relationships between environmental change and estimated biocontrol functions and by studies that directly relate traits to levels of biocontrol provision. This overview documents the novel insights into biodiversity ecosystem functioning relationships that can be derived from trait-based studies and highlights the need to understand ecosystem services from a trait-based conservation perspective.

Keywords: Agroecosystems, Araneae, Biological Control, Body Size, Carabidae, Food Webs, Predator-Prey Interactions, Traits

CO203

ARE 18 YEARS OF PASSIVE RESTORATION VIA GRAZING EXCLUSION ENOUGH FOR THE RECOVERY OF ANT COMMUNITIES? AN EXAMPLE FROM THE MONTADO ECOSYSTEM

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Grazing influences diversity and may favor the arrival of invasive species. Passive restoration, *e.g.* accomplished under grazing exclusion, is implemented in the Montado, a south-west Europe stretching ecosystem, to promote recover of key-biodiversity groups. We evaluated the success of ecosystem restoration, analyzing ant communities in a control site, with low intensity grazing, and along a chronosequence of grazing exclusion gradient, where grazing was excluded for 8, 12 and 18 years. Species richness was complemented using functional traits and groups, based on the community-weighted mean (CWM). The change on ant community composition and functional groups along the chronosequence of grazing exclusion gradient was not linear, as only the longest restored site, where ant community recovered from grazing, differed from the control. The Argentine ant occurred in all sites, except for the longest restored one, hence its presence needs to be considered to understand ant community recover. Restoration of native biotic interactions, higher species diversity and the presence of specific functional groups, might explain ant community recover in the longest restored site. Whereas more years might be needed for the re-establishment of the native ant community in the 12 and 8 years old restored sites. Taxonomic and functional homogenization characterized the control site, where the Argentine ant was the only recorded ant species. This study underlines the effects of passive restoration and the difficulties to restore sites, occupied by Argentine ant, while addressing the importance to complement species richness using functional groups to evaluate community recover.
Acknowledgements: This study was supported by Fundação para a Ciência e a Tecnologia, within the Fellowship PD/BD/114364/2016 and the project FCT-PTDC/AAG-GLO/0045/2014

Keywords: Passive restoration, Formicidae, species richness, functional traits, grazing, Argentine ants, Montado ecosystem

CO204

SPECIES TRAITS AND COMMUNITY PHYLOGENY OF CARABID COMMUNITIES ACROSS AN ENVIRONMENTAL GRADIENT FROM NIVAL BELT OF DOLOMITES TO SALT MARSHES OF ADRIATIC COAST

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Species traits of carabid beetles are increasingly used in evaluation of habitat conservation status or as indication of human impact intensity for ecosystem management, but the selection pressures at the origin of the traits are poorly investigated and this may lead to misinterpretations. From 1973 to 2016 more than 325 year samples have collected along an altitude range of 2730 m and organized in biome/landscape units (ecotopes). The aim was to analyze the changes of species traits, biogeographic characteristics and related community phylogeny of the evolutionary “pathway” of carabids in European landscapes. The data concern 425 species and base on the count of 225,000 specimen. The landscape units involved have ordered in the sequence: Alpine, Prealps, boreal conifers, deciduous biomes (beech belt and mixed forest), sclerophylls, upper and lower alluvial plain, peat bogs, standing waters, riversides, sand dunes and salt marshes. The species n/sample decreases from lowland to the alpine nival biotopes (r=0,72). The percentage of endemites is steadily low in the alluvial plain and rises starting from solid bedrocks with a maximum in the nival belt (100%) and in climax habitats (r=0,88), but drops under 30% in the boreal conifer belt. Brachypterous species concentrate in mountain forests and alpine habitats and seem strongly influenced by solid bedrock substrates, winged ones dominate in lowlands. Zoophagous species are largely prevailing in all landscapes, but specialized predators surpass generalists in mountain forests. Omnivorous and granivorous carabids prefer open lands and especially the Mediterranean biome. Spring breeders show a maximum in waterside habitats, where also the highest phylogenetic diversity (macrotaxa) has found. Biennial life cycles rise in mountain forests, late autumn breeders with adult aestivation peak in the alpine belt. Trait conservatism and adaptation to the mountain environment play both an important role in shaping the community pattern along the environmental gradient.

Keywords: Carabid beetles, species traits, biogeography

CO205

STRUCTURAL DIFFERENCES BETWEEN HABITATS IN GROUND BEETLE (COLEOPTERA: CARABIDAE) NETWORKS

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Using mathematically generated co-occurrence networks as means of detecting real-life interspecific interactions has become a frequently utilized tool of ecologists in the last decade. Yet, how accurately these networks model the complexity of ecosystems is still unclear. In this study we investigate how the structures of Bayesian Networks based on co-occurrences depend on the habitat and the assemblages they are based on. We built ground beetle (Coleoptera: Carabidae) interaction networks, partly from already published literature data, and partly from the authors own collections. Habitats were grouped into intensely managed, moderately managed and natural management types. Widely used network properties (*e.g.* degree distribution, betweennes, and connectivity) were calculated and networks from different habitats were compared using these metrics. Moreover, network species clusters were also investigated for preferential grouping based on life-traits and taxomic distances. There was a clear separation in network structure between intensely managed, and moderately managed habitats, whilst natural habitats showed similarities to both. Some natural and most of the intensely managed habitats had low connectivity, most highly connected habitats were moderately managed habitats. Proportion of strong links has increased with management intensity, whilst that of weak links has decreased. A weak taxonomic grouping was detectable amongst carabid species but only body size from the life-traits was influential in the clustering. In conclusion, the structure of Bayesian Networks were capable of showing the differences between ground beetle assemblages, and were sensitive to habitat differences. There is a demand for highly resolved networks in ecology but until we find a cheap and rapid way to map real-life interactions, network based on species co-occurrences can provide suitable surrogates.

Keywords: Carabidae, Bayesian networks, management, ecological network

CO206

CHIRONOMID SPECIES INDICATORS OF FRESHWATER ECOLOGICAL STATUS

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Chironomid species as indicators of ecological status in inland waters. Chironomids are the species richest family among macroinvertebrates and are often used as indicators of inland waters ecological condition (Armitage *et al.*, 1995). Unfortunately (or fortunately) the high number of species present has as a consequence that their taxonomic expertize is still in progress and new species are up to date described also in the well known West Palaearctic region. A review of the present knowledge is here presented, discussing the most actual problems concerning taxonomy, biogeography and ecology. The possible use of Chironomids to give answers to global warming, anthropogenic stressors, alien species invasions is considered. The data available from Italy cover a period of about 50 years and the possibility of detecting a shift in species composition is discussed. The data were filed in a Microsoft Access relational database and analysed using the R environment. Traits summarizing the species response to environmental variables are considered, with emphasis on natural and man influenced factors (water temperature, conductivity, dissolved oxygen). Species response to strict anthropogenic factors as toxic metals, pesticides etc. still remains an unresolved problem especially because of a lack of data. Our results confirm the high information content of Chironomid taxocenosis and constitute an essential component of freshwater investigation with the aim of an ecological status amelioration as requested by WFD.

Keywords: Chironomid, diversity, indicator, taxonomy, Italy

Session 7.
Genetics and Evolutionary Biology

Evolutionary Genomics

CO207

HOUSEFLIES AS MODEL FOR STUDYING TRANSITIONS IN INSECT SEX DETERMINATION

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Sex determination is a fundamental developmental process, but the underlying genetic mechanisms are remarkably diverse. Sex determination in insects occurs by a cascade of hierarchically organized genes, consisting of a primary signal, transformer (tra) and doublesex (dsx). In the housefly, *Musca domestica*, multiple sex determination mechanisms occur, including male heterogamety, female heterogamety and maternal sex determination. Male development requires the presence of a male-determining factor, M, that can be located on the Y-chromosome or on any of the autosomes. Maternal provision of Md-tra mRNA activates zygotic Md-tra and leads to female development. Mdmd induces male development by breaking the autoregulatory feedback loop of Md-tra. Some populations carry a transformer variant, Md-traD, which is insensitive to Mdmd suppression. An Arrhenogenic strain exists that is deficient in maternal tra provisioning. Transitions between these sex determination variants can be explained by structural changes in the genome and alterations in gene regulation. Further study of the polymorphic sex determination system of the housefly will be instrumental for understanding the evolutionary dynamics of insect sex determination, as well as for improving insect mass rearing and pest control.

Keywords: Sex determination, *Musca domestica*, transformer gene, sex chromosome

CO208

INSIGHT INTO THE DISTRIBUTION AND VARIABILITY OF VIRAL INTEGRATIONS IN THE GENOME OF THE ASIAN TIGER MOSQUITO, *AEDES ALBOPICTUS*

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The size and the composition of eukaryotic genomes is influenced by the amount and the type of harboured repeated sequences, collectively referred to as the repeatome. Transposable elements (TEs) are the major and most-studied components of the repeatome because of their potential mutagenic affects. Besides TEs, the repeatome includes other simple sequence repeats, such as mini- and microsatellites, as well as endogenous viral sequences (EVEs) and non- or poorly annotated DNA. Unexpectedly, recent meta- and genomics analyses have expanded the origin of viral sequences integrated into eukaryotic genomes to sequences from non-retroviral RNA viruses. Integrations from nonretroviral RNA viruses, collectively called “Nonretroviral Integrated RNA Virus Sequences” or NIRVS, appear to be mainly host-specific and tend to be found in lower numbers (<10). Like integrations from retroviruses, there are examples of NIRVS that have been co-opted for a variety of host functions, mostly leading to antiviral immunity. Despite NIRVS have been recognised as a novel component of the eukaryotic repeatome with potential immunity functions, there is limited knowledge of their widespread within and among species. To gain insight into the distribution of viral integrations and their impact on genome composition, we analysed NIRVS landscape of the genome of the Asian tiger mosquito *Aedes albopictus*. We show that *Ae. albopictus* harbours 72 NIRVS loci, which are enriched within currently-annotated piRNA clusters and have extensive variability both regarding their presence/absence across individuals and their sequence polymorphisms. These results indicated that viral integrations are an unappreciated source of genome variability.

Keywords: Repeatome, viral integrations, *Aedes albopictus*

CO209

ACTIVITY OF THE MARINER TRANSPOSABLE ELEMENT IN DROSOPHILA SIMULANS: MALES VS FEMALES

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Natural populations often have to face lasting changes in their environment, especially during the colonization of new areas. Considered as useful genetic “tools” promoting rapid adaptation to new environments, transposable elements (TEs) can also have deleterious consequences on their hosts. Indeed, the costs and benefits balance of enhanced transposition during the colonization process has rarely been analyzed. To address this question, we analysed the activity (copy number, transcriptional and excision rate) of the mariner mos1 element in natural populations of *Drosophila simulans* and put it in perspective with transcriptional variation levels of the piRNA silencing system. We found that (i) mos1 expression is always higher and more variable in testes than in ovaries; (ii) that mos1 activity is higher in colonizing populations compared to the sub-Saharan African ones (ancestral populations); (iii) that mos1 variations in transcript levels and copy number are negatively correlated with transcriptional variations of piRNA genes, aubergine and argonaute3. These results strongly suggest that the expression polymorphism of piRNA genes could be responsible for the mos1 variations, first between male and female germlines and second, according to the status of natural populations (colonizing or not). These results provide new perspectives about TEs and piRNA genes co-evolution in Drosophila germlines.

Keywords: *Drosophila simulans*, mariner, natural population, transposable element activity, colonization

CO210

GENE REGULATORY EVOLUTION UNDERLYING THE LOSS OF A POLYGENIC METABOLIC FUNCTION

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Loss of metabolic functions is one of the hallmarks of symbiosis, as functions become redundant due to provision by the symbiotic partner. Loss of function is often caused by degradation or complete absence of the underlying genes, but in the evolution of parasitoid insects lipid synthesis has been repeatedly lost without degradation of lipogenic genes. I show that regulatory changes in a few genes with low connectivity in the network likely underlie loss of this conserved metabolic trait. Using the parasitoid wasp Nasonia vitripennis, which has been shown to lack lipogenesis, a genome-wide transcriptome experiment was performed to reveal regulatory changes underlying the evolutionary loss of lipogenesis. Gene expression levels were compared between sugar-fed and starved individuals. The results show that all key genes involved in lipogenesis were transcribed at normal levels (compared to *Drosophila melanogaster*). However, changes in expression levels were discovered in a small number of key genes coding for enzymes in the parasitoid's acetyl-CoA metabolism. Together, these transcriptional modifications likely inhibit fatty acid synthesis sufficiently to prevent lipogenesis taking place. We verified this hypothesis by generating knockdown wasps using RNA interference. Our results suggest that modification of expression levels of only a few genes is sufficient to enable complete loss of lipogenesis in Nasonia vitripennis. We conclude that gene regulatory changes alone suffice to explain cases of phenotypic trait decay.

Keywords: Trait loss, lipid synthesis, gene expression, gene degradation, parasitoid

CO211

PAN-ARTHROPOD ANALYSIS REVEALS SOMATIC PIRNAS AS AN ANCESTRAL DEFENCE AGAINST TRANSPOSABLE ELEMENTS

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The small RNA pathway is an evolutionarily ancient mechanism that uses small RNA molecules bound to Argonaute proteins to regulate gene expression, combat viruses and suppress transposable elements (TEs). Due to their potent and highly-specific regulatory effects, small RNAs are of considerable interest in the development of novel pesticides, but the evolution of these pathways across different species is still relatively unexplored. In animals, small RNAs termed PIWI-interacting RNAs (piRNAs) silence TEs in the germline, preventing genomic instability and mutation. piRNAs have been detected in the soma in a few animals, but these are believed to be specific adaptations of individual species. To investigate the evolution of small RNA pathways across the arthropods, we sampled somatic and germline tissue from 20 species including horseshoe crabs, woodlice, bees and locusts. We sequenced small RNAs and transcriptomes from each tissue, and find that somatic piRNAs are common across the arthropods. By mapping this data onto the arthropod phylogeny, we show that somatic piRNAs were likely present in the ancestral arthropod more than 500 million years ago, and have been lost on multiple independent occasions. We also find that somatic piRNAs target TEs in all species, but genes and viruses are targeted in only a subset of lineages. Our results call into question the view that the ancestral role of the piRNA pathway was to protect the germline, and demonstrate that small RNA silencing pathways have been repurposed for both somatic and germline functions throughout arthropod evolution.

Keywords: Small RNA, piRNA, transposable elements, evolution

CO212

GENOME SEQUENCE OF *CAMPODEA AUGENS*, A BLIND SOIL-DWELLING ARTHROPOD BELONGING TO THE HEXAPOD ORDER DIPLURA

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The last few years have seen several sequencing projects that have greatly expanded the number of arthropod species with sequenced genomes, but genomic resources within Entognatha (Protura, Collembola and Diplura) remain very limited. In the framework of the i5k initiative, we assembled and analysed the draft genome sequence of *Campodea augens*, a blind soil-dwelling hexapod belonging to the systematic order Diplura (two-pronged bristletails). DNA was sequenced using an Illumina HiSeq 2000 machine employing four paired-end short insert libraries and four mate-pair libraries. After testing several assemblers, the final assembly was performed using the software Platanus. Contigs were scaffolded with SSPACE and gaps were resolved using GapCloser. Additional tools, namely Redundans and AGOUTI, were employed for further improving the assembly quality. The final genome size spans 1.14 Gbp, a value close to the size estimated via flow cytometry (ca. 1.2 Gbp) and harbours around 22,800 predicted genes. Contig/scaffold N50 are at 32 and 235 Kbp, respectively. BUSCO analysis indicates that the assembly contains 97% of 1,066 single-copy genes conserved across arthropods. Genomic information from species of Diplura is essential for understanding the early evolution of hexapods and their genomes, and our phylogenomic analysis indicates Diplura as a sister group to Insecta. Moreover the *C. augens* genome greatly expands our knowledge on the origin and evolution of gene families involved in perception, detoxification, and immunity in Hexapoda.

Keywords: Diplura, genome, *Campodea augens*, Entognatha, chemosensory genes

CO213

PROGRESS IN UNDERSTANDING THE EVOLUTION OF SEX CHROMOSOMES IN MOTHS AND BUTTERFLIES

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Moths and butterflies (Lepidoptera) have sex chromosome systems with female heterogamety (WZ/ZZ or derived variants). Results of fluorescence *in situ* hybridization (FISH) with genomic, W-chromosome painting, and BAC (bacterial artificial chromosome) probes along with available sequence information suggest that lepidopteran W chromosomes are almost completely composed of repetitive sequences. The W chromosomes evolve rapidly and their molecular composition differs considerably even between closely related species, as we have recently shown in the magpie moth, *Abraxas grossulariata*, an iconic species in which the female heterogamety was discovered, and its congeneric species *A. sylvata*. On the contrary, Z chromosomes are highly conserved in Lepidoptera, as demonstrated by synteny mapping of Z-linked genes between distant species using BAC-FISH and linkage analyses. The W chromosome is an evolutionary novelty in Lepidoptera, as it is absent in the sister order Trichoptera (caddisflies) and in primitive moths such as Micropterigidae. Our recent data on the W presence/absence in lower Lepidoptera, together with conserved synteny of Z-linked genes, suggest the multiple origin of the W chromosome, although its single origin followed by repeated losses cannot be ruled out. Based on these new data, we have revised the hypothesis on the origin of the W chromosome.

Keywords: Lepidoptera, Evolution, Molecular differentiation, Sex chromosomes, Synteny mapping

CO214

THE GENOME OF *DROSOPHILA SUBPULCHRELLA* AND THE EVOLUTION OF FRESH FRUIT FEEDING IN DROSOPHILA SUZUKII: A COMPARATIVE GENOMICS APPROACH

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Drosophila suzukii is an invasive fruit fly pest that is spreading across temperate regions of North America and Europe. This species is characterized by a peculiar ecology, whereby females use a serrated ovipositor to lay eggs under the skin of fresh ripe fruits on which larvae feed. The genetic bases of such ecological and behavioral traits are starting to be investigated, for instance by combining genome-wide molecular evolution studies with ecological and behavioral observations. To increase the power of these analyses, we produced a draft genome and the transcriptome of *D. subuplchrella*, the closest known non-pest sister species of *D. suzukii*. Our comparative evolutionary analyses reveal a complex evolutionary history characterised by an initial speciation no older than 2 million years ago with past and possibly ongoing genetic admixtures between the two species. Moreover, the results indicate that two species share most of the chemosensory gene repertoire, indicating a progressive modification in the suzukii subgroup toward the peculiar *D. suzukii* feeding and oviposition behaviour. Finally, we could disentangle the evolutionary dynamics of Wolbachia symbionts in the two species. Our results indicate that comparative genomics between closely related species is particularly powerful when studying species-specific adaptations and ecological traits, which could be particularly relevant when defining management strategies for invasive species.

Keywords: *Drosophila suzukii*, *Drosophila subpulchrella*, comparative genomics, evolution

CO215

EVOLUTIONARY-FUNCTIONAL GENOMICS FOR ARTHROPOD CHARACTERISATION, CONTROL, AND CONSERVATION

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The increasingly thorough sampling of arthropod diversity through genomic sequencing offers new and powerful opportunities to explore links between gene evolution and gene function in an evolutionary systems biology framework. Sequenced genomes represent a logical framework for building comprehensive biological knowledgebases that support and drive biological research. However, these cannot exist in isolation, as cross-species comparisons detail the evolutionary histories of genomic elements and so inform their putative functions. To develop the best possible hypotheses on putative gene functions we must learn how to (i) use the accumulating data to meaningfully and quantitatively characterise the full evolutionary histories of genes and other genomic elements, and (ii) relate these evolutionary histories to assayable functions and thereby pave the way to exploiting real predictive power. Recent studies that combine detailed gene evolutionary characterisations with data from large-scale functional genomics experiments exemplify how this can enhance our understanding of the biological processes being studied. Thus, to substantially advance the biological characterisation, control, and conservation of arthropods, requires the joining of forces between evolutionary and functional genomics, and this should become the norm rather than the exception.

Keywords: Evolution, function, genomics, mosquito

Evolution and Development of Functional Traits

CO216

SYMBIONTS AND PARASITES: YIN AND YANG SHAPING HOST PHYSIOLOGY

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Beneficial microbial symbionts serve important functions within their hosts, including dietary supplementation and maintenance and development of immune system homeostasis. We use the tsetse fly, *Glossina morsitans*, and its obligate mutualist, *Wigglesworthia glossinidia*, to investigate the co-evolutionary adaptations that influence multiple host physiological processes. The obligate symbiont Wigglesworthia is maternally transmitted to tsetse's intrauterine larvae, and provides a multitude of vitamins and co-factors that are essential for tsetse host physiology. We can produce flies that lack this symbiont by interfering with symbiont transmission process. Such offspring give rise to adults that exhibit a largely normal phenotype, with the exception being that they are reproductively sterile and also have a highly compromised immune system. Our results demonstrate that

Wigglesworthia in particular is required during the development of immature progeny in order for tsetse's immune system to function properly during adulthood. Tsetse flies also vector African trypanosome parasites. Tsetse midgut structures, in particular the peritrophic matrix, is a barrier that reduces parasite transmission efficiency. Our results have shown that the major surface coat protein of the parasite that is acquired from infected mammals interfere with the production of peritrophic matrix, which in turn enables the parasite to bypass this barrier and establish infections. Thus the parasites have the ability to manipulate host immune responses to achieve their transmission. Both the mutualistic endosymbionts and parasitic trypanosomes can modulate their host's physiology to benefit ultimately their transmission.

Keywords: Tsetse, symbiosis, parasitism, immune system

CO217

THE GENETICS OF VIVIPARITY IN GLOSSINIDAE: THE NOVEL GENETIC ADAPTATIONS UNDERLYING THE EVOLUTION OF LACTATION IN TSETSE FLIES

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Tsetse flies function as the sole vectors of human and animal Trypanosomiasis in sub-Saharan Africa. In addition to their role as disease vectors, tsetse flies distinguish themselves from other Diptera in terms of the amazing physiological adaptations. Of these adaptations, the reproductive biology/physiology of these flies stands out as one of the most dramatic. Female tsetse flies carry their young in utero for the entirety of their immature development and provide their complete nutritional requirements via the synthesis and secretion of a milk like substance into the uterus. Tsetse milk is derived of roughly 50% lipids and 50% proteins. The milk proteins are coded for by repurposed genes such as transferrin and acid sphingomyelinase for which orthologus sequences are found in other insects and two novel gene families for which no orthologs outside of the Family Glossinidae are known. These genes are under tissue and stage of specificity transcriptional regulation and are expressed in tight correlation with the female's pregnancy cycle. In addition, while these genes are tsetse specific, the upstream regulatory sequence from a representative gene conveys equivalent tissue, sex and stage specific reporter gene expression in transgenic Drosophila. The availability of genomic data from six representative tsetse species facilitated the comparison of these novel gene families and revealed tight conservation across species. These findings indicate that these genes are under intense purifying selection and are key components that evolved in response to the transition from oviparous to viviparous reproduction.

Keywords: Tsetse, Glossina, Reproduction, Lactation, Milk, Vector, Trypanosomiasis, Viviparity, Functional Genomics

CO218

SEXING THE TIGER, *AEDES ALBOPICTUS*

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Using morphological, cytogenetic and molecular approaches, we have developed markers that can be used to sex *Aedes albopictus* at different developmental stages, from embryos to adults. Chromosome 1 which includes two homomorphic homologues, is considered to carry the male determining factor. A sex-related banding pattern distinguishes the two homologues of chromosome 1. This banding pattern, together with FISH analysis, allows the identification of the chromosome 1 homologue carrying the *Nix* gene, the putative male-determining factor. We characterized the *Ae. albopictus* male-specific Nix locus at the gene level and in terms of its transcriptional activity in different developmental stages and in adult body compartments. The gene has two exons separated by a short intron. Given that Nix is present only in males and given its precocious transcriptional activity, it may represent a putative male-determining factor (M) like its orthologue in *Ae. aegypti*. Comparison of ~750bp sequences encompassing most of exon 1, the intron and exon 2, from individuals from five wild population samples and one laboratory strain, revealed a complete lack of variation in the Nix sequence among populations.

Keywords: *Aedes albopictus*, *Nix* gene, M factor, chromosome1 markers

CO219

DEVELOPMENTAL GENETICS OF WING PATTERNING IN BUTTERFLIES AND MOTHS

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Butterfly wing patterns form an endless template for the study of the mechanisms that generate morphological diversity. It has been long thought that the diffusion of signaling molecules with concentration-dependent outputs, or "morphogens" could explain the emergence of discrete boundaries during pattern formation. In this talk, I will present gene expression and CRISPR-based evidence that the Wnt signaling pathway is central to the establishment of pattern shapes. Importantly, the ligand gene WntA was repeatedly mapped as a locus driving adaptive phenotypic variation in wing pattern shape in several butterflies, demonstrating it truly is a favored mutational target for the tinkering of development and the elaboration of new forms. These data establish direct evidence between morphogens, pattern formation, and the subsequent diversification of forms in nature. I will also present an road map for the future study of regulatory evolution in non-traditional laboratory model organisms.

Keywords: CRISPR/Cas9, butterfly wings, pattern formation, morphogene, Wnt pathway, Hox genes

CO220

INVESTIGATING THE GENETIC BASIS OF DIFFERENCES IN MALE GENITAL MORPHOLOGY BETWEEN DROSOPHILA SPECIES

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One of the main aims of evolutionary biology is to identify the genetic basis of phenotypic differences between species. The morphology of male external genitalia evolves rapidly between species presumably driven by sexual selection. For example, the anal plates, claspers and posterior lobes exhibit striking differences in size, shape and bristle number and morphology between the males of *Drosophila simulans* clade species, which last shared a common ancestor as recently as 240,000 years ago. However, little is known about the underlying genes and how they have altered the development of these structures. To address this, we have carried out high-resolution introgression mapping of genes underlying clasper and posterior lobe size differences between *D. mauritiana* and *D. simulans*. We mapped several small regions on chromosome III, which confirmed that variation in these traits is highly polygenic, and showed that different loci underlie clasper and posterior lobe differences. Moreover, we identified a region of 177 kb containing only 9 genes that explains approximately 14% of the difference in clasper morphology between these two species. Expression and functional analyses of these positional candidates revealed that tartan, which encodes a transmembrane leucine-rich repeat protein that regulates cell interactions, is required for the regulation of clasper development and that cis-regulatory changes in this gene probably contribute to clasper size differences between *D. mauritiana* and *D. simulans*. Therefore, we have identified a new gene underlying clasper development and possibly one of the first loci underlying the evolution of genital diversification among species.

CO221

ASSESSING THE ROLE OF ACCLIMATION AND ADAPTATION IN THERMAL PERFORMANCE CURVES

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Thermal performance curves (TPC) of ectotherms, are often used to infer species responses to changes in temperature including long-term responses to climate change. Textbook examples often show temperate species to have broader TPCs and lower optimal temperatures compared to tropical species. Furthermore, theory predicts that acclimation to low temperatures shift the TPC to lower temperatures and that this plasticity is larger in species originating from variable thermal environments. Nonetheless, there are still many aspects of thermal plasticity in fitness that are poorly studied and only little empirical work have investigated theories about the evolution, shape, and plasticity of TPC's. Here we measure thermal tolerance limits and TPCs of fitness components; specifically, viability, development rate, and fecundity at seven temperatures in 22 species of Drosophila reared at a common temperature. For 10 of these species, we also measured thermal capacity and thermal performance following developmental acclimation to three additional temperatures. Using these data, we test several fundamental assumptions about the evolution and plasticity of TPCs. We found that cold tolerance and the response to acclimation varied strongly between the species in a predictable pattern. This confirms the marked effect of evolutionary adaptation and acclimation on thermal tolerance. However, contrary to expectation, for fitness traits we find that the breadth of TPCs is similar in temperate, widespread and tropical species and we also find that the plasticity of TPCs is very limited. This suggests that the thermal performance of fitness traits in Drosophila species are temporally and spatially stable. These data support the idea that thermal tolerance limits have evolved in response to extreme environmental conditions that limits species persistence whereas the temperature range for optimal thermal performance is likely under selection by the temperatures that prevail during the more benign growing season.

CO222

GENE TARGETING OF THE FEMALE DETERMINING TRANSFORMER GENE IN THE MAJOR AGRICULTURAL PEST *CERATITIS CAPITATA*, THE MEDITERRANEAN FRUITFLY: AN UNEXPECTED CRISPR/CAS9 INTERFERENCE EFFECT

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The Mediterranean fruitfly *Ceratitis capitata* (medfly) is an agricultural pests of high economic impact. We targeted medfly transformer, the key female determining gene with the ability to autoregulate, in a way similar to Sex-lethal in Drosophila. We injected Cas9-sgRNA RNPs into Ceratitis female-only XX embryos which developed into G0 adult XX flies, with up to 50% presenting a complete masculinized phenotype and exclusively a male-specific Cctra splicing pattern. However, Cctra DNA sequence analysis revealed a lack of gene editing events in both G0 and G1 progenies. We reasoned that Cctra transcription was transiently suppressed by an unplanned CRISPR interference (CRISPRi), usually achieved by a defective Cas9 variant (dCas9) unable to cut DNA. This unexpected CRISPRi seems to have caused a masculinization of XX embryos by a transient biallelic gene transcriptional silencing rather than biallelic DNA mutations. We propose that, similarly to dCas9, a wild type Cas9 protein can cause CRISPR interference in autoregulated genes that are required very early during embryogenesis. We propose that also a wild type Cas9 protein, similarly to a defective Cas9 version (dCas9), can cause CRISPR interference, in genes able to positively autoregulate.

Keywords: CRISPR/Cas9, gene editing, developmental genetics, Ceratitis capitata, sex determination

CO223

MULTIFACETED FUNCTIONS OF THE CONVERSED PI3K/AKT/FOXO PATHWAY IN REGULATION *AEDES AEGYPTI* HOMEOSTASIS

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Aedes aegypti is the main urban vector of arboviruses such as dengue, Zika and chikungunya, worldwide. For this reason, understanding the molecular mechanisms governing mosquito homeostasis is pivotal for designing new intervention tools that interferer with the mosquito life cycle and or vector-virus interactions. The PI3K/AKT/Foxo pathway has important roles in metabolism, reproduction, stress tolerance, life span and immunity in a wide range of organisms and is evolutionary conserved and functional in *Ae. aegypti*. This pathway is majorly modulated by the kinase AKT, a negative regulator, which acts by phosphorylating the transcription factor Foxo, hence preventing the activation of downstream genes. However, the repertoire of genes and mechanisms by which the pathway controls mosquito homeostasis are still largely unknown. Here, we simulated the activation of the PI3K/AKT/Foxo pathway by RNAi-mediated AKT silencing. We further used RNA-Seq global transcriptional analysis to uncover the AKT-responsive genes. Our data reveal a strong modulation of genes associated with distinct physiological processes, including innate immunity, mitochondrion activity and reproduction, such as antimicrobial peptides (AMPs), NADH dehydrogenase subunits and prophenoloxidases (PPOs). In addition, we investigated the eventual effects of AKT-silencing on the mosquito gut microbiota and found significant alterations in both bacterial load and diversity in AKT-silenced mosquitos compared to the respective controls. Especially, a clear interplay between Orders Enterobacteriales, Pseudomonadales and Burkholderiales was detected upon AKT silencing, suggesting an important role for the PI3K/AKT/Foxo pathway in controlling gut homeostasis. In summary, our study unveils the repertoire of genes and mechanisms by which the PI3K/AKT/Foxo pathway controls the mosquito homeostasis, and highlights a key putative role of the pathway in the trade-off of resources during the mosquito life cycle.

Keywords: PI3K/AKT/Foxo pathway, *Aedes aegypti*, homeostasis, microbiota, innate immunity, physiology

CO224

HORIZONTAL GENE TRANSFER CONTRIBUTES TO THE EVOLUTION OF HERBIVORY IN INSECTS AND MITES

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An evolutionary transition towards herbivory in animals is severely constrained by various hostile plant traits. Arthropods have nonetheless counteracted many of the plant nutritional and defensive barriers and are currently considered as the most successful animal herbivores in terrestrial ecosystems. We gather a body of evidence showing that genomes of a surprisingly high number of plant-feeding insect and mite species possess genes whose presence can only be explained by horizontal gene transfer (HGT). HGT is the asexual transmission of genetic information between reproductively isolated species. The genomes and transcriptomes of sexual and asexual chelicerates were also specifically screened to obtain a first insight into the deep evolutionary histories of these unique genes during chelicerate evolution. Although HGT is known to have great adaptive significance in prokaryotes, its impact on eukaryotic evolution remains obscure. Here, we show that the enzymes encoded by horizontally transferred genes into arthropods not only remain active after the transfer event, but also underpin many adaptations to phytophagy. Enzymes encoded by these genes of a foreign origin play significant roles in the arthropod assimilation pathways of plant vitamins, carbohydrates and amino acids. Additionally, arthropods also rely on horizontally transferred genes to overcome anti-herbivore plant defenses. Horizontally transferred genes and the traits they encode often functionally diversify within arthropod recipient lineages, hereby enabling the colonization of additional host plant species and organs. We demonstrate that HGT can drive metazoan evolution by uncovering its prominent role in the adaptations of insects and mites to exploit plants.

Keywords: Herbivory, horizontal gene transfer, genomic innovation

Evolutionary Ecology

CO225

INSECT IMMUNE STRATEGIES: RESISTING, TOLERATING AND REMEMBERING INFECTIONS

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Host immune defences are complex traits that are vital for individual fitness. In addition to evolving evolving defences, hosts can alter their strategy towards pathogens during their lifetime, *i.e.*, show phenotypic plasticity in immunity. Insect immune priming is one such example, and is when an individual's initial exposure to a pathogen confers enhanced protection, *e.g.* increased survival, upon a second exposure to the same pathogen. It is thought that priming improves host resistance to a pathogen, which would mean that compared to a non-primed host, a primed host has a lower pathogen load after a second exposure. Theory predicts that priming could also enhance tolerance. This would mean that primed hosts would be better at reducing the negative fitness consequences of an infection than non-primed hosts. The possibility that priming affects tolerance has not to date been tested, but it is important because resistant individuals are not necessarily the fittest. I will present data from our studies exploring whether priming is mediated by resistance and/or tolerance and whether it is dependent upon host genotype or is a more generalisable phenomenon.

CO226

MICROEVOLUTION OR WIDE TOLERANCE? BIOMARKERS OF EXPOSURE IN *SPODOPTERA EQIGUA* HÜBNER (LEPIDOPTERA: NOCTUIDAE) EXPOSED TO CADMIUM FOR OVER 150 GENERATIONS

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Populations of insects living in polluted and unpolluted environments differ significantly in tolerance to environmental stressors. Whether the differences are heritable or result from phenotypic plasticity depends, among others, on the character of stressors and duration of exposure, which may cover

many generations. Laboratory experiments revealed changes in defensive, protective and/or life history parameters of animals exposed to harmful factors. The aim of this project was to describe the stress markers in first 12 generations of *Spodoptera eqigua* Hübner (Lepidoptera: Noctuidae), an important polyphagous pest, constantly exposed to 5.5, 11, 22 and 44 µg Cd/g dry weight of food (groups Cd5.5, Cd11, Cd22 and Cd44, respectively) in comparison with the control insects and insects exposed to 44 µg Cd/g dry weight of food for over 150 generations (groups K and KCd). The concentrations of stress proteins: metallothioneins (Mts) and heat shock proteins (HSP70) were measured by means of ELISA method in the midguts of the 5th stage larvae. Mts and HSP70 concentration in the insects of Cd5.5, Cd11, Cd22 and Cd44 groups of the 1st generation were significantly higher than in both control groups. In the 2nd – 6th generations the control groups K and KCd did not differ from each other but were lower than in the groups exposed to cadmium for up to 6 generations. In the 12th generation the levels of Mts and HSP70 were homogenous in KCd, K and Cd44 while their concentrations measured in Cd11 and Cd22 groups were significantly higher than in the remaining groups. Concluding, cadmium appeared to be a selective factor. The level of Mts and HSP70 may indicate the development of increased tolerance to cadmium that, in the strongest exposure group, was detectable in the XII generation. The study has been financed by the National Science Centre Poland, grant no 2016/21/B/NZ8/00831.

Keywords: Metallothioneins, heat shock proteins, beet armyworm, cadmium, generations

CO227

MALE REPRODUCTIVE AGEING IS ACCOMPANIED BY A DECLINE IN MALE ABILITY TO PRODUCE ADEQUATE EJACULATES

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Senescence, the deterioration of physiological function and increased risk of death with advancing age, is accompanied by a loss of reproductive functions. We used the fruit fly *Drosophila melanogaster* to document reproductive ageing in males by studying known determinants of male reproductive success. Male reproductive ageing to date has been mainly explained by a reduction in sperm quality. However, in addition to sperm males also transfer seminal fluid proteins (Sfps) in their ejaculates, which cause profound changes in females upon receipt at mating and are vital for male reproductive success. We here allowed males to age up to seven weeks and then tested their ability to reproduce successfully. We particularly measured aging male ability to induce female post-mating changes known to be elicited by Sfps. As we found male ability to induce these female post-mating changes to decrease with age we then investigated age-dependent gene expression changes for five representative Sfp genes. We found a decrease in male Sfp gene expression with age that matched our findings of reduce phenotypic responses. Hence, male reproductive ageing is accompanied by a decline in functionality of the male accessory gland, the main tissue producing the majority of the male Sfps. Together our findings indicate that with advancing age male flies transfer less effective ejaculates with significant effects for male reproductive success.

Keywords: Sexual selection, ageing, sperm competition, fecundity

CO228

PARASITE-MEDIATED SEXUAL SELECTION IN MOTHS

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Theoretically, parasite-mediated sexual selection significantly contributes to the evolution of variation within and between species, and hence in the speciation process. Most of this theory has been tested in artificial systems under laboratory conditions, and both positive and negative interactions have been found: positive interactions when hosts exert terminal investment, and negative interactions when sexual signals are honest and compete for the same resources as parasite resistance. Surprisingly little data exist on how naturally occurring parasites affect sexual attraction in sexually monomorphic insects with chemical signals, which comprise the majority of species on earth, including moths. Moths (~130.000 species) are the ideal model animals to experimentally determine how parasites affect sexual attraction, as moths have a clearly defined sexual attraction, males are attracted by females through a species-specific sex pheromone, and parasites frequently occur in field populations. We recently found naturally occurring parasites on *H. armigera* in Australia as well as in the newly invaded country Brazil. This parasite, a neogregarine cf Ophryocystis, has the same life cycle as *O. elektroscirrha*, a well-known parasite of monarch butterflies. In preliminary experiments with *H. armigera*, we found that parasitized females differ in their sex pheromone quantity as well as quality compared to unparasitized females. As variation in sexual attraction determines the level and extent of genetic exchange between individuals, divergence in sexual attraction signals and responses can directly lead to extinction or speciation, and thus affect biodiversity.

Keywords: sexual attraction, sex pheromone, neogregarine, terminal investment, honest signaling

CO229

INNATE AND ACQUIRED HOST PLANT CHOICE DURING RANGE EXPANSION IN THE MOTH SPODOPTERA LITTORALIS

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To explain why there are so few generalists, *i.e.* polyphagous, insect species it has been suggested that there is a neural limitation in the amount of information that an insect brain could process. To overcome this limitation, polyphagous insects may utilise their previous host plant experience for example from the larval stage, and as adults choose the plant they developed upon themselves; the so-called Hopkins' host selection principle. Recent research has shown that the Egyptian cotton leafworm moth, *Spodoptera littoralis*, which is an extreme generalist, has an innate host plant preference hierarchy but could change the preference order between different plants and prefer the host plant that they fed on as larvae. Within a research project focusing on phenotypic plasticity during range expansion, we studied host plant preferences in a range margin population from Italy. Preliminary results indicate that ovipositing females have a different innate preference hierarchy than our lab population origin from Egypt and that they do not utilise larval experience for adult host plant choice. Thus, both innate host plant preference and plasticity in host plant choice might evolve during range expansion.

Keywords: Evolution, host plant choice, Lepidoptera, phenotypic plasticity, range expansion

CO230

DO HOST SPECIALISTS COPE BETTER WITH OXIDATIVE DAMAGE AND THEREFORE LIVE LONGER?

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Ageing is a decline in physiological function as individuals grow older, leading to decreased reproductive performance and ultimately death. Attempts to understand and explain the mechanisms of ageing are not recent and several theories have emerged, of which the free radical theory of ageing (FRTA) has received the most attention since the 1950s. The FRTA proposes that ageing results from accumulation of oxidative damage to proteins, lipids and nucleic acids that occurs when reactive oxygen species (ROS), produced by the mitochondria during aerobic respiration, overcome antioxidant capacity and repair mechanisms. This may lead to a state of oxidative stress, cellular process dysfunction, and ultimately death. Therefore, it is expected that long lived individuals or species have the best protection against oxidative damage. Recently, the role of ROS in ageing has been placed within an evolutionary context where it is suggested that ROS are a physiological constraint that mediates trade-offs between life-history traits. In line with the FRTA and given that ROS may be a mediator of the lifespan-reproduction trade-off, any environmental constraint that impacts either lifespan or reproduction should be associated with the equilibrium between oxidative damage and antioxidant capacity. In this study, we explored the evolution of ageing and correlated mechanisms by investigating how different ageing patterns among species may be shaped by host specialisation and temporal availability. To address our objectives we used eight fruit fly species from the family Tephritidae that exhibit a variety of evolutionary strategies, from extreme host specialisation to generalisation. By measuring oxidative damage to proteins and lipids, antioxidant capacity and lifespan, we show that ageing patterns in relation to the degree of specialisation in tephritids are sex-specific. Females tend to become the longer-lived sex as specialisation increases, but this is modulated by phylogenetic relationships and oxidative damage-antioxidant capacity equilibrium.

Keywords: Tephritidae, lifespan, antioxidant capacity, oxidative stress, host specialisation

CO231

PREDATORS AND PHENOTYPES - CAUSES AND PATHWAYS OF DIVERSITY

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Proof for predation as an agent shaping evolutionary trait diversification is accumulating, however, our understanding how multiple antipredator traits covary due to phenotypic differentiation is still scarce. Species of the dragonfly genus Leucorrhinia underwent shifts from lakes with fish as top predators to fishless lakes with large dragonfly predators. Here, we show how a variety of traits evolved in response to changes in the selective regime with high multicollinearity among variables suggesting that traits have evolved in concert rather than independently when Leucorrhinia shifted from fish lakes to dragonfly lakes. Our results highlight the importance of predation as a factor affecting patterns of multiple trait covariation during phenotypic diversification.

Keywords: Antipredator traits, dragonflies, habitat gradient, predation, trait diversification

CO232

LIFE-HISTORY EVOLUTION IN A BUTTERFLY – FROM GENES TO METAPOPULATION DYNAMICS

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Organisms are constantly challenged by environmental variation, for example in resource quality, which subsequently influence life history variation and evolution in natural populations. We are studying life-history responses and underlying coping mechanisms to environmental stress, namely changes in host plant quality induced by drought, in the Glanville fritillary (*Melitaea cinxia*) butterfly. With effectively coupling laboratory and field based studies, we show how developmental time, resulting body mass as well as subsequent adult fitness-related traits are shaped by variation in food quality. We show that some of the responses are developmental stage-dependent, and that coping mechanisms include developmental switches as well as behavioural adjustments in both larvae and adults. Variation among families suggests genetic variation in the observed responses, which are further assessed by on-going genomic analyses. Our specific questions are assessed within an ecologically relevant context, as there is spatial and temporal variation in summer drought and host plant quality, as both temperature and precipitation conditions from spring to late summer greatly impact the metapopulation dynamics of the butterfly, and finally as the preference of warm microclimatic conditions by the female butterflies places the offspring and the local populations at risk of extinction during hot summers. Working with the large metapopulation of the Glanville fritillary butterfly hence gives us a unique opportunity to assess the processes operating from genes within individuals all the way to metapopulation-level dynamics.

Keywords: Evolutionary ecology, life history, genotype-phenotype association, metapopulation, environmental stress

CO233

THE EVOLUTIONARY GENETICS OF INDUSTRIAL MELANISM – AN UNFOLDING STORY

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Polymorphism for the degree of melanism in several species of moth, and spatio-temporal variation in the frequencies of these morphs with respect to the regional history of coal smoke pollution, provides an opportunity for studying the mechanism of recurrent adaptation in natural populations. I will describe what we know so far about the genetics and evolutionary dynamics of this polymorphism in different regional populations of Biston betularia, and British *Odontopera bidentata* and *Apocheima pilosaria*.

Session 8.
Insect Control Biotechnology

Use of RNA Interference for Insect Control

CO234

ENCAPSULATION OF DSRNA WITH GUANYLATED POLYMERS INCREASES RNAI EFFICIENCY IN *SPODOPTERA EXIGUA*

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Exploiting the RNA interference (RNAi) mechanism to target essential genes for knockdown in pest species, without harming other organisms in the ecosystem, is one of the most promising novel pest control strategies under development. Lepidoptera, which comprise some of the most devastating agricultural pests, are known to be refractory to oral RNAi due to a very high capacity for nucleolytic degradation of dsRNA in the intestinal tract. This is partly accommodated by a very alkali environment in the gut. In this research, we developed novel guanidine-containing polymers to protect dsRNA against nucleolytic degradation, specifically in high pH environments. First, an *ex vivo* approach was used to select the polymers with the best protection capacity at a high pH. Next, *in vivo* assays were performed, feeding chitin synthase B dsRNA which was complexed with the best performing polymer (high guanidine content) to *Spodoptera exigua* larvae. We observed a significantly higher gene silencing at the transcript level and a more than 3-fold increase in mortality in the polymer-dsRNA treatment (53%), compared to the naked dsRNA treatment (16%). We also observed that the polymer-dsRNA complex treatment completely halted the development of the treated larvae, while larvae feeding on naked dsRNA had a relatively normal development. Finally, confocal microscopy studies, using labelled polymers and dsRNA were performed to confirm successful cellular uptake of the dsRNA or complexes in lepidopteran midgut CF203 cells. To our surprise, we observed an increase in cellular uptake efficiency for the polymer-dsRNA compared to the naked dsRNA. This new delivery strategy could be of great use in further fundamental research in lepidopterans, using RNAi as a research tool, and could lead to future applications for RNAi-based pest control of lepidopteran insects.

Keywords: RNAi, Lepidoptera, polymer, nanocarrier, Spodoptera

CO235

DSRNA COMPLEXATION WITH BLOCK COPOLYMERS ENABLES PEST CONTROL BY ORAL APPLICATION

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Without significant advances in crop-protection, it is likely that food production will not be able to sustain an ever-increasing world population. Unfortunately, environmental concerns and the increasing frequency of pesticide resistance means our current arsenal of insecticides is unlikely to be sufficient. As a result, it is important to consider alternatives to existing chemical pesticides. Biopesticides derived from natural sources with new modes of action, such as peptide/protein toxins, neuropeptides, insect hormones and double-stranded RNA (dsRNA) mediated RNA interference (RNAi), have been proposed as alternatives. RNAi provides sequence specific suppression of gene expression, which can be utilised for insect pest control. RNAi mediated by dsRNA has a number of positive attributes over conventional chemical insecticides, such as its selectivity for a target pest. However, the efficiency of RNAi varies from species to species, and its wide-scale use is currently limited by its rapid degradation upon ingestion and suffers from poor uptake from the gut. By complexing dsRNA to specific block copolymers containing a complexing and stabilising block, we can increase the protection of the dsRNA to degradation from extracellular nucleases and increase RNAi efficiency. Specifically, we have produced well-defined diblock copolymers comprising poly(2-hydroxypropyl methacrylamide) –b-poly((dimethylamino)ethyl methacrylate) (PHPMA-b-PDMAEMA) which are subsequently quaternised. We have investigated their complexation with dsRNA. Phenotypic observations of the increased stability and penetration was undertaken for both complexed and naked dsRNA targeting the expression of a *Drosophila suzukii* gene. It was found that upon complexation of the dsRNA, a 75% increase in mortality was achieved compared to naked dsRNA fed to this important fruit pest. Feeding of the complex to a close relation *D. melanogaster* had no adverse effects on development and survival, demonstrating the selective nature of this complexed dsRNA. Our approach has the potential to greatly improve the efficacy of dsRNA as a bioinsecticide

Keywords: Biopesticide, Controlled delivery, *Drosophila suzukii*, RNAi

CO236

TECHNOLOGIES TO CONTROL, SURVEIL, AND COUNTER GENE DRIVES

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Site-specific endonucleases can be engineered to spread desirable traits throughout wild population for the control of vector-borne diseases. We have previously shown proof of principle of genetic technologies aimed at eliminating malaria-transmitting mosquitoes by biasing the reproductive sex ratio toward males or using gene drive to spread genetic modification interfering with mosquito reproduction. Currently, there are limited options available to robustly control the activities of gene drive nucleases and mosquito-based gene drives. We propose new approaches that will enable temporal and spatial control of gene drive nucleases such as RNA-based post-transcriptional regulators, tools for countering nuclease-based gene drives as well as novel strategies to surveil for their mutagenic activities and genome-wide off-target detection.

Keywords: Gene drive, malaria, mosquito, genetic vector control, CRISPR, genome editing

CO237

GENOMIC AND TRANSCRIPTOMIC ANALYSIS OF THE OLIVE FLY REPRODUCTIVE SYSTEM

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The olive fruit fly, *Bactrocera oleae*, is the most important pest of cultivated olives causing significant production losses and olive fruit impoverishment. Currently, olive fly control mostly relies on insecticide spraying. However, the extensive use of insecticides leads to resistance development and environmental damages, rendering the design of new alternative methods of control a necessity. Targeting the reproductive success of any insect is a promising method for pest control as a possible manipulation of the reproductive system could affect its destructive activity. The present study focuses on the genomic and transcriptomic analysis of the olive fly reproductive system and the potential to utilize this knowledge towards the fly's population control. Initially, a comprehensive analysis of the reproductive system was performed focusing on the identification of genes related to post-mating responses. Specifically, RNAseq was performed for reproductive tissues of virgin and mated insects. Comparison of the transcriptomes resulted in the identification of genes that are differentially expressed after mating. The top 100 most highly differentially expressed genes from each comparison were further annotated to the newly sequenced olive fly genome and functionally annotated through the Gene Ontology database. Annotation showed an alteration in metabolic, catalytic and cellular processes in the mated tissues. Moreover, functional analysis through RNAi silencing was performed for selected genes. Specifically, yellow-g and troponin C genes were successfully silenced through injections of dsRNA and sex peptide receptor (spr) was successfully silenced through feeding of dsRNA producing bacteria. To clarify if the transient silencing of the genes had an impact on reproduction, we carried out mating experiments that showed significantly reduced oviposition rate of the tested insects. In conclusion, the first comprehensive analysis of the reproductive system of B. oleae revealed genes that could be target for the development of new intervention methods.

Keywords: Reproductive system, olive fly, *Bactrocera oleae*, RNAi, dsRNA, transcriptomics

CO238

GENE SILENCING IN *TRIBOLIUM CASTANEUM* AS A TOOL FOR THE TARGETED IDENTIFICATION OF CANDIDATE RNAI TARGETS IN CROP PESTS

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A promising new agricultural technology for insect pest control is the application of RNA interference (RNAi), a post-transcriptional gene silencing mechanism mediated by double-stranded RNA. However, the number of RNAi targets that cause robust lethality in agricultural pest insects is limited. In this study, a selection of lethal RNAi target genes from the *Tribolium castaneum* iBeetle screen were used to demonstrate efficacy of orthologous targets in the economically important coleopteran pests western corn rootworm (*Diabrotica virgifera virgifera*) and European pollen beetle (*Meligethes aeneus*). We analyzed transcript orthologs of 50 selected *T. castaneum* genes in *D. v. virgifera* diet-based RNAi bioassays; 21 of these RNAi targets resulted in lethality and 32 showed growth inhibition. Low dose injection- and diet-based dsRNA assays in *T. castaneum* and *D. v. virgifera*, respectively, enabled the identification of the four most potent RNAi target genes: Rop, dre4, ncm, and RpII140. Transgenic corn plants engineered to express RNA hairpins of the prioritized candidates Rop, dre4, or RpII140 showed protection to *D. v. virgifera* larval feeding damage. Knock-down of Rop, dre4, ncm, and RpII140 also showed high levels of mortality in M. aeneus both by injection and feeding. Taking together, our results illustrate the feasibility of using high throughput systems for model organisms to identify potent RNA targets for difficult-to-work with agricultural insect pests.

Keywords: RNA interference (RNAi), insect pest control, *Tribolium castaneum*, *Diabrotica virgifera virgifera*, rootworm, *Meligethes aeneus*

CO239

CHLOROPHYLL DEGRADATION IN A LEPIDOPTERAN PEST *SPODOPTERA LITTORALIS* IS MEDIATED BY A GUT-SPECIFIC CHLOROPHYLLIDE BINDING PROTEIN

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Chlorophyll is a natural pigment from plants that is synthesized and conjointly degraded in an enormous number. Chlorophyll is well studied in plants whereas there are limited findings on other organisms. As insect herbivores are organisms that naturally consume leaves and thus chlorophyll, they provide a good start to learn the degradation mechanism of Chlorophyll in non-plant organisms. Our previous studies using the notorious lepidopteran pest species, the Egyptian cotton leaf worm (*Spodoptera littoralis*) have shown that i.) Regurgitate has catalytic activity to degrade chlorophyll and ii.) Catabolites were detected inside the gut and frass (Chlorophyllide, Pheophorbide, and Pyropheophorbide). In the present study, the suspected liable protein – Chlorophyllide binding protein (CHBP) – has been identified using transcriptome and proteome analysis from regurgitate of *S. littoralis*. A similar gene was also found in other lepidopterans, such as *Bombyx mori* and *Helicoverpa amiverga*. First experiments on the gene expression level in different developmental stages and tissues indicate that CHBP is mainly expressed in the digestive tract in the 5th instar. Furthermore, transcript silencing via RNAinterference was applied to larvae and indicating a metabolite change as detected by LC/MS. We also found a lower survival rate in larvae injected with gene specific dsRNA, where gene expression was decreased up to 80%. Heterologous expression of CHBP in insect cells will reveal the substrate of CHBP *in vitro*, whereas the protein purification from regurgitate will show comprehensively the enzyme activity in each fractions. This study may have an impact in herbivore-plant interactions considering CHBP's role in survival rate, prospectively it would be of importance in agriculture to fight against lepidopteran pests.

Keywords: Chlorophyll degradation, chlorophyll metabolites, RNAi, lepidopteran

CO240

RNAI-BASED BIOPESTICIDES

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Over the past decade, RNA interference (RNAi), the sequence-specific suppression of gene expression triggered by specific dsRNA molecules, has proven to be a very promising strategy in crop protection. The main advantages of RNAi are its selectivity, as well as the lack of persistency in and damage on the environment as a whole. In this paper, we report on the promising results against pest insects such as the western corn rootworm *Diabrotica virgifera*. Also successes have been reported against other beetle pests as Colorado potato beetle, but also sucking pest insects as Asian citrus psyllids and mites. In addition, a number of challenges will be discussed that needs to be addressed to implement RNAi as a widely-used pest control strategy. One of these challenges is a variable efficiency. While some insects show a very robust, efficient and systemic RNAi response, many others show a limited or variable RNAi response. Possible causes for this variability in sensitivity are degradation of the dsRNA in the insect body, insufficient uptake into the cells, viral interactions or problems with the RNAi machinery in the cells.

Keywords: RNAi, pest control

CO241

LITERATURE REVIEW OF BASELINE INFORMATION ON RNAI THAT COULD SUPPORT THE ENVIRONMENTAL RISK ASSESSMENT OF RNAI-BASED GM PLANTS

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As part of a procurement form the European Food Safety Authority (EFSA), a systematic literature search was performed to collect all available peer-reviewed studies on RNAi in invertebrate species (Nematoda, Arthropoda, Mollusca and Annelida) to provide a baseline information on this technology that could support the environmental risk assessment of RNAi-based GM plants. With this literature search, we retrieved a total of 5,076 publications. Based on this database, an overview was compiled of all studies using oral delivery of small RNAs (sRNAs) to these invertebrates. This overview includes information on tested species, life stage, sRNA molecule type, target gene, concentrations used, outcomes, etc. A second part of our assignment was to provide several narrative reviews on different topics such as environmental and cellular uptake of sRNAs, RNAi efficiency and factors involved in sensitivity, possible exposure routes of small RNAs to (non-)target organisms, potential unintended effects by sRNAs on invertebrate species in the agroecosystem and also on the availability and use of genomic data in risk assessment of RNAi-based GM crops. Here, we present an overview of the literature search, the conclusions of the narrative reviews and we provide some exemplary case studies.

Keywords: Genetically modified (GM) plants, RNA interference (RNAi), systematic literature search, invertebrates, biosafety, specificity, bioinformatics

CO242

IDENTIFICATION OF LETHAL RNAI TARGETS AND THEIR APPLICATION IN RELEVANT PEST SPECIES

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One of the most challenging problems in crop protection is the increasing tendency of pest insects to develop resistance to various insecticides, thus raising a demand for alternative control strategies. Silencing of genes by RNA interference (RNAi) appears to be one of the most promising methods under intense investigation for pest control purposes in agriculture. RNAi is a universal gene-silencing mechanism in eukaryotes which is triggered by endogenous or exogenous double-stranded RNA molecules (dsRNAs) and evolved as a line of defense against viral infections. Some insect species are able to elicit an RNAi response upon oral up-take of dsRNA, which is a prerequisite for RNAi-mediated crop protection. However, the main limitation of RNAi-mediated pest control is the identification of suitable candidate genes that kill the pest after dsRNA ingestion. Recently, a number of lethal RNAi target genes has been identified in the red flour beetle, *Tribolium castaneum*, which served as templates to identify orthologous genes in pest species. The main focus of our work is the identification of such orthologs in the coleopteran pest species *Leptinotarsa decemlineata* and *Phaedon cochleariae* and to assess their efficiency upon oral ingestion under realistic exposure scenarios. Furthermore, we aimed to transfer our findings to other pest organisms including Noctuid moth species. The paper reviews the recent identification of lethal target genes in a model coleopteran, and describes prospects, limitations and failures associated with an RNAi based approach to control pests of agricultural importance.

Insect-Associated Microorganisms: Their Possible Role for Biotech Application

CO243

EXPLOITATION OF WOLBACHIA TO CONTROL THE TRANSMISSION OF EMERGING AEDES ALBOPICTUS-BORNE HUMAN DISEASES

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In recent years, the endosymbiotic bacterium Wolbachia pipientis acquired a leading role in the context of the biotechnological tools available for the development of innovative control strategies against mosquito vectors. This success is mainly due to two biological properties shown by certain strains of this bacterial endosymbiont: i) the ability to induce a post-mating mechanism of reproductive incompatibility, known as Cytoplasmic Incompatibility (CI); ii) the induction of a reduced vector competence for various severe viruses once an appropriate Wolbachia infection has been established in suitable vectors. Combining the exploitation of these two properties can be the basis for a control strategy aiming at exploiting the expected modifications in reproductive biology and vector competence of a target species to contribute to reduce its epidemiological role. This objective was pursued focusing on *Aedes albopictus* through the replacement of its native Wolbachia infection with specific foreign strains. Herein, we review all of the results achieved by this research and discuss them in the context of developing opportune strategies of field application. Specifically, we highlight the potential of a method of *Ae. albopictus* suppression based on the exploitation of Wolbachia also pointing out the needed safety and sustainability issues. Furthermore, we analyze the opportunity to promote a strategy of disease control based on the replacement of the wild-type *Ae. albopictus* with populations characterized by reduced vector competence. These approaches are discussed with the support of both theoretical and experimental data.

Keywords: *Aedes albopictus*, Wolbachia, Incompatible Insect Technique, Population replacement

CO244

IMPACTS OF THE MOSQUITO GUT HOMEOSTASIS ON MALARIA TRANSMISSION

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Malaria parasites enter in the mosquito gut upon blood feeding on a human gametocyte carrier, where they reproduce and grow into motile forms before they can invade and traverse the gut epithelium to establish infection. Detail understanding of the mosquito gut microbiota and homeostasis can lay the foundations for novel methods for malaria transmission control. Mosquito blood feeding triggers a significant, transient increase of the gut microbiota, but little is known about the mechanisms by which the mosquito controls this bacterial growth whilst limiting inflammation of the gut epithelium and prevents systemic infection. Our up-to-date understanding of these mechanisms in the mosquito *Anopheles gambiae* and their impact on malaria infection will be presented and discussed. These include the spatiotemporal changes in the gut microbiota community upon blood feeding, recognition of bacteria and subsequent immune responses, regulation of mosquito physiology including synthesis of the peritrophic matrix, and direct and indirect impacts in malaria infection and transmission.

Keywords: Anopheles, Plasmodium, malaria transmission, gut microbiota, gut homeostasis

CO245

SODALIS GLOSSINIDIUS AS A DELIVERY SYSTEM FOR NANOBODIES THAT TARGET THE TSETSE-TRYPANOSOME INTERPLAY

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Sodalis glossinidius, a secondary bacterial symbiont of the tsetse fly, is currently considered as a potential delivery system for anti-trypanosomal components interfering with the capability of the tsetse fly vector to transmit parasitic trypanosomes (*i.e.* paratransgenesis). Recently, we demonstrated the proof-of-concept that Sodalis can be genetically engineered to express and release significant amounts of functional anti-trypanosome Nanobodies (Nbs) in different tissues of the tsetse fly. Next, we developed novel strategies for i) establishing stable chromosomal expression in Sodalis allowing strong and constitutive expression of anti-trypanosome Nbs in the absence of antibiotic selection, ii) the sustainable colonization of the fly and its subsequent generations with genetically modified Sodalis through microinjection of the bacterium into third-instar larvae and iii) studying the transmission dynamics of modified Sodalis into the following generations of progeny. In this study, we engineered Sodalis to express different Nbs that target the *T. brucei* procyclic stage and tested their ability to block *T. brucei* development in the tsetse fly midgut. One of these Nbs (*i.e.* Nb_88) significantly compromised the parasite development in the tsetse fly midgut whereas another one (*i.e.* Nb_19) exerted a significant infection-enhancing effect at the level of midgut establishment. These data are the first to show the ability of Sodalis to deliver *in situ* effector molecules that are able to target the trypanosome-tsetse fly crosstalk, representing a valuable contribution to the development of a paratransgenic tsetse fly based control strategy. Finally, Sodalis-based Nb delivery could also be of interest as a powerful *in vivo* laboratory tool to unravel the molecular determinants of the parasite-vector association.

Keywords: Paratransgenesis, tsetse, Sodalis, Nanobodies

CO246

LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) USING FRASS FOR SURVEILLANCE AND EARLY DETECTION OF *AGRILUS PLANIPENNIS* INCURSION/INFESTATION IN CANADA

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The Emerald Ash Borer (EAB), *Agrilus planipennis* Fairemaire, is considered one of the worst alien forest pests to ever invade North America. To date, EAB has killed or infested approximately 100 million ash trees since it was first detected and ash trees in Canada and the US are at risk of imminent infestation and death. To substantiate EAB infestations in ash trees, it is essential to obtain adult or larval specimens to perform morphological or molecular analysis. Nonetheless, obtaining such specimens from traps and infested trees can be a daunting task. Hence, a diagnostic tool that is non-intrusive for the trees and able to be performed in the absence of any insect developmental stage is crucial to confirm EAB infestations within suspected ash trees. Our molecular detection method exploits the detectability and accessibility of frass/gallery material, and the accuracy and rapidity of the LAMP technology. Species-specific LAMP primers were designed with the partial sequence of the cytochrome c oxidase subunit I region of EAB. The developed EAB-LAMP assay showed high specificity to detect EAB DNA with no cross-detection of closely related Agrilus species and all probed wood boring beetles. The sensitivity of the EAB-LAMP assay was validated using field collected frass/larval gallery material and the optimized EAB-LAMP assay was considered adequate for practical on-site use. Our LAMP assay also successfully detected EAB adult DNA from trap captures that contained other Agrilus species. Since the developed LAMP assay is cost-effective, easy-to-operate, rapid, highly sensitive and specific, it will contribute to the early detection of new incursions and accurate identification of A. planipennis during routine surveillance. Similar approaches may be used as an early warning system to implement timely measures to prevent the spread and possible eradication or containment of alien invasive insect species.

Keywords: EAB, invasive species, cytochrome c oxidase subunit I, loop-mediated isothermal amplification, molecular identification

CO247

A ROBUST AND EFFICIENT CRISPR-CAS9 MUTAGENESIS PROTOCOL IN THE PEA APHID

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CRISPR-Cas9 has emerged in the last few years as a very efficient functional analysis tool in model and non-model organisms. Aphids damages on crops are largely explained by their unusual life cycle, since they reproduce most of the time asexually (by parthenogenesis during spring and summer), sexual reproduction only occurring at the end of autumn, in response to photoperiod shortening. Sexual individuals thus mate and lay eggs that over-winter through an obligatory 3 months diapause. In most of insect species where sexuality is the only reproductive mode, mutagenesis protocols rely on the injection of fertilized eggs to generate stable and germline transmissible mutational events. Aphids thus represent a very challenging model since sexual reproductive mode switch must be triggered by carefully controlled photoperiod conditions. Here we developed a step-by-step CRISPR-Cas9 mutagenesis protocol in the pea aphid by targeting Stylin-1, a cuticular protein gene likely to be involved in phytovirus vection. To achieve that, we injected fertilized eggs with a mixture of single guide RNAs (targeting coding sequences of the gene) and Cas9 protein. The overall procedure takes almost 7 months from photoperiodic induction of sexual morphs (2 months), mating and eggs injection (2 weeks), obligatory diapause for surviving eggs (3 months), lineages hatching (3 weeks) and germline transmission evaluation in their offspring (3 weeks). We could first estimate directly after injection an in-ovo mutation rate of nearly 70%. Then after diapause hatching rate of melanized eggs was about 8% ended up in total with 16 established lineages producing offspring. Among those, 6 lineages showed stable mutations of one or both alleles. Germline transmission rate of mutations was thus around 37.5%. Despite the peculiarities of the biology of the aphid our study clearly demonstrates the efficiency of the CRISPR-Cas9 mutagenesis protocol in this insect. These stylin-1 edited aphids represent so far the first mutant lineages ever generated and open exciting opportunities to eventually demonstrate and validate gene function in this major crop pest.

Keywords: Pea Aphid, CRISPR-Cas9, photoperiod induction, egg injection

CO248

TRANSGENIC FUNGI PREVENT MOSQUITOES FROM TRANSMITTING MALARIA PARASITES

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We have tested the efficacy of a mosquitocidal Metarhizium strain (Met-Hybrid) engineered to express an insect-specific, spider neurotoxin (Hybrid) and GFP under the control of a hemolymph-specific promoter. The experiments are being conducted under semi-field conditions in a multi-chambered MosquitoSphere (a contained, near-natural environment) constructed for this purpose in a region of Burkina Faso where malaria is endemic. We found that suspending Metarhizium in locally produced sesame oil and spreading this suspension on black sheets achieves a long-term effect in the sphere, and we are developing alternative applications of these fungi. Compared to a strain of wild-type virulence expressing RFP (Met-RFP), Met-Hybrid killed anopheline mosquitoes in half the time and at much lower spore doses, which increased the percent of lethally infected mosquitoes and the effective persistence of the pathogen. We also demonstrated that Met-Hybrid had important pre-lethal effects that included reduced blood feeding by infected mosquitoes and synergistic control of insecticide resistant mosquitoes. This NIH-funded, international effort represents an important step in the progression of transgenic mosquito control technologies into field application. We are currently working on community engagement for an eventual open field release to test epidemiological and clinical impacts of transgenic Metarhizium. Our results will have broad implications for any project proposing to scale up new, complex, and potentially controversial technologies for malaria eradication.

Keywords: *Anopheles gambiae*, Metarhizium pingshaense, Vector Biology, Malaria, Transgenics

CO249

OBLIGATE INTRACELLULAR PATHOGENS IN NATURAL POPULATIONS OF *OSTRINIA SPP*. IN SOUTH-WESTERN RUSSIA

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Stem borers of the genus *Ostrinia* is an attractive model of insect ecology, physiology, evolution and pathology. Two sibling species, *Ostrinia nubilalis* and *Ostrinia scapulalis*, exist as sympatric populations on most of Europe, occupying similar biotopes but feeding on different host plants, monocotyledonous and dicotyledonous, respectively. *O. nubilalis* is also a dangerous pest of maize. Obligate intracellular parasites are important for insect population dynamics, though little is known about their distribution in natural populations of *Ostrinia*. During a more than 20 years of monitoring over *O. nubilalis* population dynamics, two depression pits were observed, each followed by a baculovirus epizootic, suggesting that activation of viruses is a consequence and not the cause of the host depression. Molecular genetic identification using conserved genomic loci (lef-8 and polh) revealed multiple genotypes, either previously known (such as *Mamestra brassica* multiple nucleopolyhedrosis virus) or novel (with 88% sequence similarity to known species). To reveal cryptic infections caused by Wolbachia and microsporidia, molecular genetic screening was performed using a collection of genomic DNA extracts from sympatric populations of *O. nubilalis* and *O. scapulalis*. Significantly higher rates of Wolbachia prevalence in insects from mugwort and hemp (*O. scapulalis*) as compared to those from maize (*O. nubilalis*) were revealed in three out of four localities. Because *O. nubilalis* and *O. scapulalis* are genetically isolated and Wolbachia is vertically transmitted, it can be suggested that pathogens from two host populations are not exchanged and their dynamics patterns are not therefore correlated. The distribution of Microsporidia was more even and didn't differ significantly between the populations belonging to these two different species. Since microsporidia are spread both vertically and horizontally and easily cross interspecific barriers, their exchange between populations of closely related hosts is likely to occur.

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Keywords: baculoviruses; microsporidia; Wolbachia; *Ostrinia*

CO250

EFFECTS OF NATURAL TEMPERATURE REGIMES ON DIFFERENT WOLBACHIA INFECTIONS IN *AE. AEGYPTI*

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Controlled releases of *Ae. aegypti* infected with Wolbachia bacteria are currently considered one of the biggest effort for suppression of arboviruses worldwide. Wolbachia is a maternally transmitted endosymbiont known to interfere with replication of RNA viruses, including Dengue and Zika, and can invade populations through mechanisms of reproductive manipulation. Nevertheless, the ability to persist in the field strictly depends on environmental conditions including temperature fluctuations, which are particularly extreme in endemic areas. We investigated how thermal stress affects Wolbachia infections by exposing larvae of *Ae. aegypti* infected with different Wolbachia strains (wMel, wAlbB and wAu) to diurnal cyclical temperatures, mimicking the natural average temperatures of larvae breeding sites in tropical areas. This heat regime dramatically reduced wMel density within its adult host, as well as altered its tissue tropism; moreover, maternal transmission between generations was largely reduced. Similarly, these temperature cycles also reduced wAu density, although without causing transmission leakage between the mother and the offspring. In contrast, wAlbB remained stable under thermal stress, in terms of density in adult mosquitoes and of maternal inheritance. As the strength of Wolbachia-mediated virus blocking is positively correlated to its density, we challenged *Ae. aegypti* Wolbachia-infected lines with Semliki Forest Virus (SFV), comparing heat-treated and control groups. Preliminary qRT-PCR results of whole bodies indicate the virus blocking ability of heat-treated wMel and wAlbB is incomplete, however there is a higher viral load in the SFV-positive wMel mosquitoes compared to wAlbB. The wAu strain largely retains its virus-blocking ability even with heat-treatment. These results highlight that effective control strategies must be tailored to include several crucial aspects, including environmental conditions, in particular in the case of Wolbachia-based interventions, whose success deeply depends on the choice of the strain showing the highest density stability.

Keywords: Wolbachia, arboviruses, symbiosis

CO251

INSECT SYMBIONTS AS BIO-CONTROL AGENTS AGAINST PLANT PATHOGENS

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Phloem restricted bacterial pathogens pose a major threat in many agricultural crops. Because the conventional application of chemical sprays is inefficient, endophytes have been suggested as a potential reservoir for innovative control approaches. We hypothesized that insects that are involved in the transmission of plant pathogens may harbor microbes that affect disease agents. The study reported is focused on a bacterium that was isolated from the planthopper *Hyalesthes obsoletus* (Hemiptera: Cixiidae), the insect vector of Bois noir disease. The isolate belongs to a novel bacterium from the family Xanthomonadaceae, for which the name *Frateuria defendens sp.* nov. is proposed. This isolate could be introduced into a number of healthy and infected crop plants, and its presence in plant tissues was confirmed up to four weeks post inoculation. In the presence of the isolate symptoms of disease-causing bacteria such as Phytoplasma and Liberibacter were markedly reduced in both laboratory and field experiments. Taken together, the results demonstrate that insects that serve as vectors for plant pathogens may be a useful source for potentially beneficial bacteria.

Keywords: Endophyte, Liberibacter, phytoplasma

Biopesticides, Genetically Modified Organisms and Maintenance of Ecosystem Services: Can They be Profitably Integrated?

CO252

MODELLING AN ADAPTIVE RESISTANCE MANAGEMENT PLAN FOR SESAMIA NONAGRIOIDES IN BT-MAIZE

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The possible evolution of resistance by target pests is one of the threats for a sustainable use of Bt-crops. Insect resistance monitoring plans aimed at early detections of possible onset of resistance are usually undertaken by the applicants where Bt-crops are cultivated. The most commonly adopted strategy for delaying the occurrence of resistance is the high dose-refuge strategy, in which sufficient refuges of non-Bt host plants near Bt-crops have to be provided. Several features linked to the biology of the target organism and the receiving environment are the major drivers for resistence allele frequency evolution. Simulation models indicate that insect resistance can be delayed considerably by the high dose-refuge strategyand nearly all high-dose toxins have remained effective against their targeted pests where IRM plans have been correctly implemented. The recent data collected in Spain about allele frequency of *Sesamia nonagrioides* indicate that while current Bt-maize varieties are still resistant against this Lepidopteran pest, their resistant allele frequency is increasing. We have used a simulation model to evaluate the possibility of implementing an adaptive management plan. According to the results of the model, a sensitive annual sampling plan aimed at detecting an occurrence <0.03 allele frequency in the population of the target insect will give time enough to implement contingency plans before resistance occurs in field. However, in the case of very high adoption rates of Bt-maize, a drastic reduction in planted areas will be needed to significantly delay resistance. Implications for resistance management plans of S. nonagroides will be discussed.

Keywords: Adaptive resistance management, Bt-maize, Simulation models

CO253

ENGINEERING INVASIVE Y CHROMOSOMES FOR MOSQUITO CONTROL

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Selfish sex chromosomes have been identified in a number of species, since their existence within a population - manifesting as extraordinary sex ratios in the offspring of individuals carrying them. Sex chromosomes harboring alleles that favorably bias their transmission to the next generation act to selectively eliminate gametes bearing the opposite sex chromosome. As a result, both the allele (sex distrorter) and the sex chromosome that harbors it increase in frequency in a population over time until the population either becomes extinct or until resistance alleles arise that counteract the activities of the sex distorter. Beyond their captivating significance for biology and evolution, the adverse effect that such selfish sex chromosomes could have on population fitness has been considered for its application in the control of pest species, particularly insects. Synthetic genetic constructs can now be engineered to behave similarly to naturally occurring sex distorters. Towards this end, we are building invasive Y chromosomes in malarial mosquitoes that could spread within natural populations, dramatically reducing the frequency of biting females and thereby diminishing the reproductive capacity of the population, leading eventually to collapse. We will present our recent findings related to the identification of YG5, an *An. gambiae* Y duplication of an ancestral topoisomerase with testis specific expression. We will also present the YG5 role in the engineering of *An. gambiae* Y chromosome for mosquito vector control.

Keywords: gene drive, sex chromosome biology, population control, selfish genetic elements

CO254

EFFICACY OF GARLIC ESSENTIAL OIL-BASED NANOEMULSION IN CONTROLLING TWO CROP KEY INSECT PESTS

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The wide-scale use of synthetic chemicals is still the most influential pest management tool worldwide. Yet, insecticides have multiple undesired effects, *e.g.* production cost increase, side effects on non-target organisms and on the environment, pest resistance, human health risks. Thus, sustainable practices are strongly encouraged by public opinion and policy makers. Research efforts are oriented toward reducing the use of synthetic pesticides with natural molecules (biopesticides) with improved safety profiles. Encouraging results have been recently obtained testing botanicals, namely essential oils (EOs), against crop, stored product and medical/veterinary pests, and microbial pathogens. Laboratory trials were conducted to assess the insecticidal activity of garlic EO, formulated as nanoemulsion, against the cotton aphid, *Aphis gossypii*, and against eggs, larvae and adults of the South American tomato borer, *Tuta absoluta*. The potential toxic effects on tomato plants were also investigated. The tested EO was analysed by a gaschromatograph coupled with a triple quadrupole mass spectrometer (GC-MS-MS). Both the zeta potential (ζ) and particle size distribution of the formulation were assessed. More than 100 volatile compounds were identified and quantified. The EO was dominated by sulfur-containing compounds, particularly allyl polysulfides. Indeed, diallylsulfide, –disulfide, -trisulfide and –tetrasulfide accounted for more than 80% of the total composition. The formulations developed had nanometric scale dimensions (180 nm) and good stability over time (ζ: -23.2mV). The nanoemulsion caused a higher contact toxicity at lower concentration for the tomato borer eggs compared with the cotton aphid, indeed the estimated LC90s were 4.41% and 16.14% for the two target pests, respectively. The bioactivity of garlic EO against *T. absoluta* was also confirmed at LC50 by causing 86.67±9.09% of larval mortality through ingestion and a significant oviposition deterrent effect in both choice and no-choice tests. Conversely, the nanoformulation did not cause any apparent phytotoxicity on tomato plants, thus suggesting a good potential for spray applications.

Keywords: Tomato, aphid, IPM, nanopesticide, *Tuta absoluta*, botanical

CO255

SUSTAINABLE USE OF TRANSGENIC BT-MAIZE: EXPERIENCE AFTER 18 YEARS OF CULTIVATION IN SPAIN

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Transgenic maize expressing the insecticidal protein Cry1Ab from *Bacillus thuringiensis* (Bt-maize) has been continuously cultivated in Spain since 1998. A surface of 124,228 ha was cultivated in 2017, which accounted for over 95% of the total Bt-maize growing area in the EU. The Ebro basin, in northeastern Spain, is the region with the highest adoption rate (more than 60%) to prevent recurring damage by the Mediterranean corn borer, *Sesamia nonagrioides* (Lefèbvre) (Lepidoptera: Noctuidae) and the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae). Since 1998, resistance monitoring programs have been implemented for these two key maize pests. Our results indicate no shifts over time in their susceptibility to the Cry1Ab protein. Data on the biology, genetics and physiology of *S. nonagrioides* paved the way to explore the possible reasons using evolutionary models. The implementation of the high-dose/refuge strategy, the EU policy decision to replace Event176 with MON810 Bt-maize and the compliance of Spanish farmers with refuge requirements has proved to be vital for the sustainability of this technology. Post-market environmental monitoring field studies have also been performed to assess possible effects of Bt-maize on non-target fauna. No detrimental impacts have been found on canopy, on-plant and ground-dwelling herbivores, predators and parasitoids inhabiting Bt-maize crops, or in the soil micro-arthropod community. The only exception was a punctual decrease in the abundance of rove beetles detected in Bt-maize in some areas and years, although laboratory studies did not evidence negative effects of Cry toxins on this group. The data compiled from these field studies have been used for the identification of representative taxa to be used as surrogates for environmental monitoring purposes.

Keywords: Biotechnology, GMO, environmental monitoring, resistance, non-target organisms

CO256

MODELLING THE TRANSGENE PERSISTENCE OF A SELF-LIMITING AUTOSOMAL SEX-RATIO DISTORTER IN A WILD-TYPE *ANOPHELES GAMBIAE* POPULATION

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Malaria transmitted by anopheles mosquitoes causes a phenomenal burden in terms of human mortality and morbidity. The development of genetically modified mosquitoes and their subsequent field releases offered innovative and feasible approaches to reduce the transmission of malaria. Recently, synthetic autosomal sex-ratio distorter with improved fitness have been developed in *Anopheles gambiae*. Activation of destabilized I-PpoI homing endonuclease during spermatogenesis induced the shredding of xchromosome during male meiosis in the testes and led to the development of the fully fertile Ag(PMB)1 transgenic line that produced ~ 95% male offspring. Although this strain can efficiently suppress wild-type mosquito populations in small cages, laboratory trials with semi-field settings should be established to predict long-term population suppression effectiveness of sex-ratio distorters and their persistence in the field. Thus, we developed and improved an appropriate modelling framework to estimate the PMB1 transgene persistence in wild-type mosquito population. We divided our study into three main steps: (1) Model parametrization by generating life history data of wild-type and transgenic Ag(PMB)1 mosquito strains, partly within a large-cage setting; (2) Modifications of the modelling parameters and running three models sequentially; (3) Model validation comparing experimental data under semi-field conditions to the expected parameters. Therefore, we performed a four-month experiment in large cages studying the dynamics of PMB1 transgene in age distributed, generation-overlapping populations of *An. gambiae*. Our data demonstrated that PMB1 sex-ratio distorter is in fact a self-limiting construct and is likely to disappear rather quickly in the field. The model validation analysis confirmed that the development of mathematical models that incorporate population age structure (*e.g.* assortative mating by age) is crucial for predicting long-term success of transgenic strategies. Our results also highlighted that mating behavior and food searching mechanisms had a major effect on mosquito longevity in large cages changing the expected PMB1 trangene dynamics.

Keywords: Population age structure, genetic vector control, homing endonuclease, large cage study, malaria, transgene frequency,

CO257

EVALUATION OF AEGEROLYSIN PROTEINS FROM THE FUNGAL GENUS *PLEUROTUS* AS POTENTIAL BIOINSECTICIDES FOR CONTROLLING COLORADO POTATO BEETLE AND WESTERN CORN ROOTWORM

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Aegerolysin proteins from the fungal genus *Pleurotus* have recently been found to interact with ceramide phosphoethanolamine (CPE), a membrane sphingolipid specific to invertebrates. Additionally, the genomes of these fungi harbour nucleotide sequences that encode proteins with a membrane attack complex/ perforin (MACPF) domain. When combined, aegerolysins and MACPF proteins can perforate artificial and biological membranes that contain the specific lipid receptor, by forming transmembrane pore complexes. Several bacterial aegerolysin protein complexes have been reported to have selective insecticidal properties, and have been successfully introduced into plants via genetic transformation. Accordingly, our aim was to investigate whether similar aegerolysin complexes deriving from *Pleurotus* spp. exhibit potential as biopesticides. Three recombinant *Pleurotus aegerolysins*, namely ostreolysin A6 (OlyA6), pleurotolysin A2 (PlyA2) and erylysin A (EryA), and their MACPF-protein partner pleurotolysin B (PlyB) were purified and characterized. The insecticidal properties of these aegerolysins, either alone or in combination with PlyB, were studied on Colorado potato beetle (CPB; Leptinotarsa decemlineata) larvae, Western corn rootworm (WCR; *Diabrotica v. virgifera*) larvae and beetles, spotted wing drosophila (*Drosophila suzukii*) flies, mealworms (*Tenebrio molitor*), grain aphid (*Sitobion avenae*) and greater wax moth (*Galleria mellonella*) caterpillars. OlyA6/ PlyB, PlyA2/ PlyB and EryA/ PlyB complexes have shown a selective toxic effect to CPB and WCR, but not to other tested insect pests. Exposure of CPB to leaf disks treated with protein mixtures significantly increased larval mortality and decreased larval feeding during the 5–day experiment. Exposure of WCR to artificial food containing OlyA6/ PlyB resulted in a significant increase of larval and beetles' mortality during the 5- and 7-day experiments. The ability of aegerolysins from the fungal genus *Pleurotus* to target CPE, and to form transmembrane pores with PlyB, suggest their potential use as biopesticides for controlling CPB and WCR.

Keywords: Aegerolysin, biopesticide, ceramide phosphoethanolamine, Colorado potato beetle, *Diabrotica v. virgifera*, *Leptinotarsa decemlineata*, maize, *Pleurotus*, potato, Western corn rootworm

CO258

OPTIMAL TEMPERATURE AND LARVAL AGE OF *SPODOPTERA FRUGIPERDA* (SMITH, 1797)(LEPIDOPTERA: NOCTUIDAE) FOR PRODUCTION SYSTEMS OF *BACULOVIRUS SPODOPTERA* (SFMNVP) - ISOLATE 6 NR

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Victor Costa, Federal University of Lavras, Brazil
Fernando Valicente, Embrapa Maize & Sorghum, Brazil

Baculovirus is a major entomopathogen to control agricultural pests. The objective of this study was to identify the temperature and larval age of *Spodoptera frugiperda* that results in an increase of mortality and number of pib using droplet feeding assay. The bioassay was conducted using 0.25µL containing 1.14x10^5 pib/larvae with *Baculovirus spodoptera* (SfMNVP) – Isolate 6 NR purified by sucrose gradient. 6 and 7-day old S. frugiperda larvae was infected and maintained at 25°C and 26°C. Every 24 hours during 12 days, dead larvae were collected and stored at -20°C. Each dead larva was weighed and the number of pib/larvae was counted. 6-day old larvae showed higher mortality of 100% at 25°C than 93.75% at 26°C, while 7-day old larvae showed 25% and 50% of mortality, respectively. 6-day old larvae showed the highest number of dead larvae at 26°C within 7 days (62.5%) after infection, however, 7-day old larvae within 8 days showed 31.25% of mortality after infection. 7-day old larvae showed a higher average

weight of dead larvae of 0.118725 g at 25°C and 0.121813 g at 26°C than 6-day old larvae. The number of pib/larvae was higher in 7-day old larvae, $1.6x10^9$ pib/larvae at 25°C and also $1.6x10^9$ pib/larvae at 26°C, than 6-day old larvae. The total number of pib was higher in 6-day old larvae at 25°C, $9.5x10^9$. Our data suggest that 6-day old S. frugiperda larvae increased mortality at 25°C with a high number of total pib produced. These results are relevant to large scale production systems, increasing its production with low number of pib/larvae and less days of infection.

Keywords: Baculovirus, biological control, biological pesticide

CO259

THE USE OF BACULOVIRUS AND *BACILLUS THURINGIENSIS* BASED BIOPESTICIDES IN BRAZIL

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The use and research with baculovirus and *Bacillus thuringiensis* (Bt) based biopesticides has increased since 2012/13, when *Helicoverpa armigera* was officially detected in Brazil. Embrapa Maize and Sorghum Research Center has a Bt and baculovírus, with more than 4.600 strains of Bt and more than a 100 baculovírus isolates, collected from different regions of Brazil since 1984. Many of these insect pathogens have been tested against: *Spodoptera frugiperda*, *S. cosmioides*, *S. eridania*, *Helicoverpa zea*, *H. armigera*, *Chrysodeixis includens*, *Dichelops sp* e *Diatraea saccharalis*. Some NPV baculovirus have been developed as biopesticides (WP-wetable powder) to control fall armyworm, soybean cartepillar and the cotton bollworm. *Baculovírus spodoptera* (isolate 6NR) is very efficient and doesn´t cause the liquefaction of the integument, and this is an important factor in a large scale production system. Baculoviruses were isolated from H. armigera, and identified as HearNPV with a CL50 of $4,1x10^4$ and formulated as WP. *B. thuringiensis* strains were found many *cry* genes (1, 2, 3, 4, 5, 6, 8, 9, 12, 13, 14, 22 e 51) e *Vip* genes(1, 2, vip3Aa1, vip3Ah1, vip3Ae1, Vip3Ba1, vip3Aa2, vip3Af1). These Bt strains were tested agains caterpillars and sucking bugs. Some Bt isolates also have been developed as biological pesticides to control fall armyworm, soybean looper and cotton bollworm. Some biopesticides were registered to be used in the field.

Keywords: Biopesticides, *cry* genes, baculovirus, *Bacillus thuringiensis*

Session 9.
Insects and Global Food Production

Feeding the World: How to Control Insect Pests of Crops Without Damaging the Planet

CO260

INVASIVE CROP PESTS: THREAT AND THEIR IMPACT ON GLOBAL FOOD PRODUCTION

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Climate change and global warming have fostered the invasive behavior of pests through the world. Some of these insect pest species cannot adapt to their new environment, some establish themselves in moderation while others adapt rapidly and thrive, creating to an enormous potential for damage. We have seen many different insect species belonging to nearly all insect orders being invasive and problematic when entering new continents. Many of the most harmful invaders belong to the Lepidoptera. Invasive Lepidoptera are already a huge problem in many agricultural areas, but their damaging potential may still increase enormously in future. This is not only true for one special pest species, nor is it limited to just one continent: There are many examples from around the world, where the invasions have occurred in recent years, for example *Tuta absoluta* in Europe and Asia, *Helicoverpa armigera* in South- and North-America or *Spodoptera frugiperda* in Africa. Which exceptional threats are posed by these species? Do they have pre-adaptions facilitating their establishment in their new environments or is it likely that they are going to adapt quickly to their new circumstances? How are we facing up to these threats today and what will our potential tools to combat such threats in future? This presentation briefly discusses options to address risks posed by invasive pests on crop-yields in selected geographies.

Keywords: Invasive insects, Food, Climate, *Tuta absoluta*, *Helicoverpa armigera*, *Spodoptera frugiperda*

CO261

ARE THERE ANY REAL ALTERNATIVES TO BT TOXINS?

Richard H. Ffrench-Constant, University of Exeter, UK

Despite considerable effort to find novel alternatives to Bt there remain surprisingly few viable alternatives to these highly successful toxins. This talk will review progress on some of the alternatives such as the 'Toxin complexes' or 'Tc's' and the class of toxins related to 'Makes caterpillars Floppy' or 'Mcf'. The central thesis of the talk will be that although toxins are often named after the species of bacterium from which they were initially isolated (*e.g.* 'Bt' for *Bacillus thuringiensis*) in fact bacteria readily transfer these toxins between them. Therefore the bigger picture that is emerging is one of a 'mosaic' in which different bacteria can possess any insecticidal toxin regardless of their species status. The implications of this new mosaic model for the biology of different bacteria and the potential use of these toxins in GM plants will be discussed, using the Tc's and the Mcf-like toxins as specific examples.

Keywords: Insecticidal toxins, Bt, Tc's, Mcf

CO262

A NEW SPECIES OF THE GENUS PSEUDOSTIGMAEUS (PSEUDOSTIGMAEUS SORGHUMUS) STIGMAEIDAE: PROSTIGMATA: ACARI FROM PAKISTAN

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Muhammad Qayyoum, Assistant Professor, Pakistan
Muhammad Farooq, Assistant Research Officer, Pakistan

Mites of family Stigmaeidae are well known predators against phytophagous mites, lepidopterous eggs and small soft bodied insects. A random survey was conducted to explore the predatory mite fauna from Punjab, Pakistan. The holotype female (male unknown) of this new species of genus *Pseudostigmaeus* was collected from city Dera Ghazi Khan on Sorghum bicolor and described here. Fifteen (15) paratypes were also collected from same collection data and seven were from Muzaffargarh. The description and illustration of main body parts, host ranges and comparison remarks with other species are also given. All specimens were deposited in the Acarology Research Laboratory-I, Department of Entomology, University of Agriculture, Faisalabad, Pakistan.

Keywords: Stigmaeidae, *Pseudostigmaeus*, Acari, new species, predatory mite

CO263

THE PROMISE OF 'PUSH-PULL' FARMING SYSTEM FOR GREEN REVOLUTION IN AFRICA

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The 'push-pull' system (www.push-pull.net) effectively controls serious biotic constraints to cereal production in Africa— insect pests (stemborers, fall armyworm) and parasitic striga weed, while improving soil health and biodiversity. The companion cropping system, makes smallholder farms more resilient often with a tripling of yields. It involves attracting insect pests with trap plants (pull) whilst driving them away from the main crop using a repellent intercrop (push). Chemicals released by intercrop roots induce abortive germination of the noxious parasitic striga weed. The companion plants provide high value animal fodder, facilitating milk production. Furthermore, soil fertility is improved due to the nitrogen fixing intercrop and soil degradation is prevented. Recently, the push-pull system has been adapted to drier and hotter conditions linked to climate change by identification and incorporation of drought tolerant companion crops (http://www.push-pull.net/Climate-smart_Push-Pull.pdf). This climate-smart push-pull directly responds to rising uncertainties in Africa's rain-fed agriculture due to the continent's vulnerability to climate change. The new companion crops, Desmodium intortum and Brachiaria Mulato II hybrid, can withstand extended periods of drought stress with no water. To date push-pull has been adopted by about 200,000 smallholder farmers in eastern and southern Africa whose maize yields have increased from about 1 t/ha to 3.5 t/ha. Low-input technologies that address several production constraints and deliver multiple benefits are more relevant for African smallholder farmers but also have useful lessons for agricultural systems in the developed world.

Keywords: Push-Pull, striga, stemborers, fall armyworm, cereal-livestock integration, climate change, soil fertility

CO264

CROP PROTECTION: THE EXTENT OF THE GLOBAL PROBLEM

Stuart Reynolds, University of Bath, UK

How will the world be fed in 2050? Pre-harvest and post-harvest crop losses impose an important restraint on our efforts to feed a growing global human population. In this paper, I compare world crop losses due to insect pests with the increase in crop production that will be required in just over 30 years' time. Extensification of agriculture (*i.e.* using more land) to increase production poses severe ecological difficulties (*e.g.* biodiversity losses), so that intensification (ie higher yields per hectare) will necessarily be an important part of the solution to the problem. This includes increased crop protection efforts and the infrastructure to make them possible. These efforts must avoid the collateral environmental damage that has plagued crop protection in the past, if for no other reason than such damage limits crop protection's success. Future crop protection problems will be most severe in developing countries because this is where extra food will be required, and where existing crop protection efforts are least effective. To be maximally effective, introduction of new crop protection techniques cannot be considered separately from existing agronomic practices. It's evident that (i) current crop protection is not very effective on a global scale; (ii) new, more effective, environment-friendly methods of crop protection must be found if world hunger is not to grow; (iii) these new technologies must be easy to apply in low-tech agricultural environments; (iv) greater investment in crop protection research and education are urgently required to deliver these outcomes.

Keywords: Crop protection, pests, crop losses, research, education

CO265

THE PROSPECTS FOR GM-IMPROVED BIOLOGICAL CONTROL FUNGI

Raymond St. Leger, University of Maryland, USA

We have applied genomics and DNA technology to insect pathogenic fungi in order to study infectious processes in general, and to provide new possibilities for solving insect pest problems. We have engineered narrow host range fungi that incorporate genes for scorpion and/or spider toxins, thereby greatly increasing their ability to kill some of the worst agricultural and disease vector pests. We have also used genes from other fungi as well as extremophile bacteria in order to greatly increase fungal survivability in host environments. In field trials with varied transgenic fungi, we have showed that their application to corn seeds boosts yield by >35%. We are currently investigating long-term issues such as the evolvability of transgenic microbes, and the needs, feasibilities, and realities of monitoring, detecting and culling engineered microbes that escape or are deliberately released into the environment. We are also working, particularly in Burkina Faso, with developing integrated control strategies based on community engagement, in order to translate laboratory discoveries to the field.

Keywords: Transgenics, pathogens, biocontrol, mosquito control, agricultural pests, Fungi

CO266

USING INSECT BIOTECHNOLOGY TO CONTROL INSECT PESTS

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Insects that feed on crops or stored products are still the most important competitors for human nutrition, and insects that transmit infectious diseases such as malaria threaten human health on a global scale. The negative environmental impact of insecticides causes increasing public concern and growing hurdles for their approval. Insect Biotechnology has been defined as the development and application of biological methods to translate insects or their molecules, cells, organs and associated microorganism into products and services for applications in medicine, industry and plant protection. This emerging field in applied entomology seeks to explore innovative and sustainable strategies to control pest and vector insects, including the engineering of transgenic plants expressing insecticidal proteins or metabolites as well as RNAi-based and epigenetic approaches. Recent advances in the sterile insect technique, which relies on the mass production and release of sterilized insects to reduce the wild-type population through infertile matings, were driven be the design of novel genetic and transgenic systems enabling radiation-free sterilization, sexing and labeling of pest insects. Insect antenna-based biosensors for *in situ* detection of volatiles have been developed to optimize sustainable insect control strategies such as the application of pheromones in plant protection. The presentation will provide an overview about selected frontiers in biotechnology-based approaches for insect pest management.

Keywords: Insect Biotechnology, Pest management, Sustainable plant protection, RNA-interference, Sterile insect technique,

CO267

UPDATE ON CONTROL OF PINK BOLLWORM; A MAJOR COTTON PEST IN PAKISTAN

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The reported diversity of pink bollworm-specific parasite species found in Pakistan supported the idea of its Indo-Pakistan origin. In 1983, Bt cotton was introduced to solve the bollworm complex problem in several cotton growing areas of the world, however, soon after in 1996 pink bollworm (PBW) developed resistance against Bt cotton. During 2015-16 there was a sudden reduction up to 14.1% cotton cultivation acreage that reduced production by 22% in Pakistan. There are different limiting factors responsible for this decreased production including severe attack of bollworm complex particularly by PBW on cotton. PBW caused 13.58% and 37.5% yield losses in Bt and non-Bt cotton, respectively. Pakistan then had great difficulty in disposing of its lint in the international market as the crop was badly affected by the frequent attacks of PBW. While, more recent data (2016-17) revealed that PBW was the sole cause for the loss of five million bales of cotton that account approximately up to 125 billion rupees. There are several reasons behind the failure to control PBW in Pakistan but most important is the leftover bolls that contain diapausing larvae serving as a source of pink bollworm infestation in the next season. Some argue that climate changes *i.e.* weather conditions favor the regular outbreaks of PBW. Reports also suggest that during 2014, Bt cotton was found susceptible to PBW due to presence of low levels of toxins expressed in the Bt crop. While farmers claimed that adulteration of seed held was responsible for PBW outbreaks. The serious decline in the production of cotton in Pakistan attracted the attention of government and they invited research organizations and industry to join hands to counteract this insect pest. The experts from different parts of world were invited to share success stories in their countries and also memoranda of understandings have been signed for providing expertise. Recently, huge funding is provided to all leading research organizations to improve the cotton and look into other advanced strategies for its control.

Keywords: *Pectinophora gossypiella*, cotton, yield, loss, decline, economic importance

Entomophagy: Eating Insects as a Contribution to Global Food Production

CO268

MORPHOLOGICAL AND FUNCTIONAL CHARACTERIZATION OF THE LARVAL MIDGUT OF *HERMETIA ILLUCENS* (DIPTERA: STRATIOMYIDAE), A PROMISING INSECT FOR BIOCONVERSION AND FEED PRODUCTION

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The Black Soldier Fly (BSF), *Hermetia illucens*, is among the most promising insect species for the reduction of organic waste. The high nutritional value of the larvae and pupae makes them an interesting and sustainable protein source for the production of animal feed. Despite the great interest toward this insect, the current literature mainly provides information on the rearing methods for BSF, and indications on its use for waste treatment and feed production, while little is known about *H. illucens* biology. In particular, a deep understanding of the physiology of the midgut, which is implicated in food digestion and nutrient absorption, is essential to better comprehend the extraordinary dietary plasticity of the larva, which is able to grow on different substrates. In the present study, we provide a detailed structural and functional characterization of the midgut of last instar larvae, by using morphological, biochemical and molecular approaches. Our results demonstrate that the larval midgut is composed of three distinct anatomical regions with different luminal pH. The midgut epithelium is formed by different cell types that accomplish nutrient digestion and absorption, acidification of the midgut lumen, endocrine regulation, and growth of the epithelium. A detailed characterization of the activity of enzymes involved in nutrient digestion and their mRNA expression levels reveals that protein and sugar digestion is associated to specific regions of this organ. According to the collected evidence, we propose a functional model of the larval midgut of *H. illucens* in which each region is characterized by peculiar features to accomplish specific functions. This work represents the first morphofunctional characterization of the midgut of *H. illucens* larvae and sets the stage for the best exploitation of the bioconversion ability of this insect.
Work supported by Fondazione Cariplo (Insect bioconversion: from vegetable waste to protein production for fish feed, ID 2014-0550).

Keywords: Bioconversion, digestion, *Hermetia illucens*, larval midgut

CO269

FIRST EVIDENCE OF A HACCP MANUAL FOR EDIBLE INSECTS IN ITALY

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In November 2015, the technological developments and an increasingly pressing scientific opinion led to the approval of Regulation (EU) 2015/2283, which effectively abolished the old Regulation (EC) 258/97, simplifying the authorization procedures of Novel Food. The subsequent EFSA Opinion (EFSA Journal 2016; 14 (11): 4594) has effectively legitimized the possibility that, even in Europe, insects may be destined for human consumption with the condition that their production observes the European rules of food safety. The large-scale use of insects as ingredients for food is today technically practicable and many industries, in various parts of the world, are already engaged in this production. In this note, we intended to formalize a first Italian contribution to the guidelines for food safety in the breeding process of edible insects, in particular for the cricket *Acheta domesticus*. The manual provides information on breeding procedures, based on the principles of the HACCP system, validated by a specific sampling plan carried out by the local health company (AUSL of Bologna). The test breeding was specifically set up at the Microvita farmhouse in Crespellano (BO). A risk-based protocol and specific procedures of insect breeding have been developed, in order to settle the best control methods applicable to the various stages of the process and highlight the critical points according to the hazards considered. The study concerned in particular the microbiological, biological, chemical and physical hazards during the production of insects and their transformation into a fresh edible product, in addition to the correct packaging, storage, and processing. This research is the first step, but it becomes essential to develop a type of insect farm that is effectively competitive, energy efficient and safe for human nutrition, leading to the creation of healthy and tasty products able to compete with traditional meat farms and be an alternative protein source.

Keywords: Edible insects, *Acheta domesticus*, HACCP, Novel food

CO270

ANTIHYPERTENSIVE EFFECT OF *TENEBRIO MOLITOR* (COLEOPTERA: TENEBRIONIDAE) DEFATTED LARVAE IN SPONTANEOUSLY HYPERTENSIVE RATS

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Hypertension is a major risk factor for many pathologies like coronary artery disease, stroke, heart failure, atrial fibrillation, peripheral vascular disease, vision loss, chronic kidney disease, and dementia. An optimal pressure value can be attained with lifestyle changes or medication, if necessary, with a significant reduction of risk of stroke, ischemic heart disease and mortality from cardiovascular diseases. A moderate control of blood pressure can be obtained by a nutritional approach and food with Angiotensin Converting Enzyme (ACE) inhibitory properties may be a valid strategy. In the last few years many peptides with ACE inhibitory activity were detected in animal and vegetal proteins after enzymatic hydrolysis, and even some insects have been also tested. ACE inhibitory activity in the mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae)(TM) protein hydrolysates derived from the larval and pupal stages have been recently described. Consequently, the antihypertensive properties of defatted TM larvae were assessed *in vivo*. Spontaneously hypertensive- (SHR) or normotensive Wistar Kyoto- (WKY) rats were feed with standard laboratory rodent chow containing fish proteins or supplemented with TM or captopril for 4 weeks. Results showed that the general health status of TM-treated rats did not differ from that of animals fed with the standard diet, both registering a constant and comparable weight gain during the 4 weeks of treatment. Furthermore, a significant reduction in blood pressure, heart rate and glycemic levels was observed in TM-feed rats, which was accompanied by neuroprotective effects. The obtained results lay the foundation for further studies on the potential of the insects protein component as a source of bioactive peptides for functional foods or nutraceuticals.

Keywords: Insects, antihypertensive effects, spontaneously hypertensive rats

CO271

INSECT BASED FEED AND ANIMAL HEATH

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Edible insects have great potential as sustainable source of valuable nutrients for animal feed. They are rich in proteins and fats and provides vitamins and minerals. Research highlighted how insect-based proteins could efficiently be included in animal feeds in partial substitution of conventional proteins commodities in fish, poultry and pigs. In addition, insects also contains different bioactive compounds having functional properties able to modulate livestock immune response and microbiome. For example, the chitin that forms the insect exoskeleton could increases the activity of the animal's innate immune system and have a protective role against infections. Chitin also modulate the microbiota with positive effects on animal performances and health. Similarly, the medium chain lauric acid (C12:0) contained in high levels in some insects fats is well known for its antibacterial effects and its ability to reduce the inflammatory process. Insects could also be a source of antimicrobial peptides providing great hope due to the global problem related to the increasing resistance of bacteria to antibiotics. Future research should then be addressed to evaluate the real potential of insects to contribute not only as nutrient source for feed but also as source of important bioactive compounds having positive effects on growth, health and resistance against pathogens.

Keywords: Insects, Livestock health, Chitin, Lauric acid, Antimicrobial peptides

CO272

GUT LACTIC ACID BACTERIA AS PROBIOTIC ADDITIVES FOR MASS REARING OF TENEBRIO MOLITOR

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Insect production for food and feed around the world is growing. With it, comes new challenges associated with mass rearing of livestock species. As such, there is a growing need for timely diagnosis, management and prevention of emerging and existing diseases. One hypothesized solution is the introduction of beneficial probiotic bacteria to the diet of the insects. In this study, we evaluated the inhibitory potential of lactic acid bacteria, extracted from the gut of *Tenebrio molitor*, on selected pathogens. We also carried out *in vivo* assessment of the fitness benefits of the bacteria to the mealworm larvae. Preliminary results point out to highly successful inhibition of bacterial pathogens *in vitro*. The evaluation of parameters such as early larval survival, growth rate and weight after the addition of probiotic formulations should allow us to discuss the potential for probiotic additives to improve industrial mass rearing facilities of the mealworm *T. molitor*.

Keywords: Insects, food, rearing, probiotic, bacteria, fitness

CO273

EVALUATION OF BIOLOGICAL PARAMETERS AND THE LIPID PROFILE OF BLACK SOLDIER FLY (HERMETIA ILLUCENS, STRATIOMYIDAE) REARED ON BYPRODUCTS FROM DIFFERENT FOOD CHAINS

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The total amount of bio-waste produced annually in the EU by the food and beverage value chains is estimated at 37 Mtons. The possibility to use insects for the valorization of byproducts from these chains may represent a sustainable and valuable solution. This study aimed to analize the development parameters and the lipid content of black soldier fly (*Hermetia illucens*, Stratiomyidae) prepupae reared on byproducts obtained from different food chains. The substrates used for this experiment were: beer brewery byproducts, tomato skins and seeds, spent ground coffee, grape stalks and marcs, bread dough, cow milk whey and fish-waste material. *H. illucens* larvae were reared on each substrate within glass containers at 28±0.5°C and 98% relative humidity. Biological parameters such as larval weight, prepupae weight and development time were recorded. Obtained prepupae were frozen before fractionation and chemical characterization. Lipid extraction was carried out using the Folch method, adjusted for the specific samples. The total lipid content was calculated and the quantitative characterization of the fatty acid profile was performed through GC-FID analysis. The best larval performance was obtained when specimens were reared on byproducts from beer, tomato and cheese food chains. The different substrate composition was reflected in the lipid profile of *H. illucens* larvae. In particular, the saturated to unsaturated fatty acid ratio showed marked changes according to the substrate. The high content and type of lipids, together with the proteins and chitin extracted from the prepupae represent high-value biobased products that might be used in the feed/food industry as well as for the development of innovative biomaterials for different technological purposes. These results suggest that mass rearing of *H. illucens* larvae, especially on some of the tested byproducts, may be exploited for an alternative employment of the food/beverage bio-waste.

Keywords: Food byproducts valorization, insect fractionation, insect composition, insect mass rearing, larval performance, feed/food industry

CO274

EATING INSECTS? PREFERENCES AND STRATEGIES OF INSECT PROCUREMENT AND SELECTION AS HUMAN FOOD IN EAST ASIA, AMAZON AND WESTERN COUNTRIES

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To feel disgust in tasting or even looking at insects and small invertebrate animals is rather a rule than a normal behavior of the Western peoples. And absent or minimal is the disgust proved towards invertebrates by most tropical for instance, Amazonic and Asiatic peoples. Being object of continuous landing of unpleasant mosquitos, flies, and similar, most Neotropical populations living the forest or the river borders learn very early the lessons from the parasites however they balance these pests blames with the rewards they can obtain from a number of invertebrates they eat. But not all eat the same species, among different groups in Amazon for instance. And there is a strategy that favor invertebrate categories feeding on the more renewable portions of the forest, the ones linked to leaves and litter are the more relevant insects. Another attitude seems the one in which the insects are consumed from plants providing edible fruits as well. In the current wave that support the insects as the new gourmet food in Western, is quite important consider two points: the primitive disgust we have towards invertebrates that need to be removed properly and the food-web that will be utilized to mass produce it. These food-web must be sustainable, alternative to the ones for humans and clean.

Keywords: Edible insects, traditional food, mini-livestock, Amazon, Asiatic countries.

CO275

THE CONTRIBUTION OF INSECTS AS FOOD AND FEED TO GLOBAL FOOD SECURITY

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The need for alternative protein sources is urgent as available agricultural land is not sufficient to satisfy the growing demand for meat. To use insects as food and feed is a promising alternative and the interest worldwide to engage in it is growing exponentially. This is testified not only by the growing number of start-up companies, but also by the increasing number of published scientific articles. The attention is triggered by the many advantages that insects as food and feed have to offer in comparison to conventional meat and feedstuffs: low greenhouse gas emissions, high feed conversion efficiency, low land use and adequate nutritional quality. Insect can also contribute to a circular economy as they are able to transform low value organic side streams into high value protein products. In tropical countries more than 2000 insect species are eaten, but now there is a lot of attention in the western world to develop nutritious insect-based food products. Nutritional values of edible insects are similar to those of conventional protein sources, and there are even health benefits for both humans and animals. Insects can also be used as feedstocks to replace the current used products such as fishmeal and soy, which are becoming increasingly expensive. The feed industry will require reliable huge quantities of these products, which should be of constant and safe quality. Industrial automated rearing facilities for a number of species are already being developed. Insect as “sentient beings” have not received much attention but recent findings suggest that this may not be justified. Research needs to address nutritional benefits, food safety concerns, environmental impact, optimizing farming, consumer acceptance and gastronomy. When legislation becomes more conducive, insects as food and feed have the potential of becoming an important new sector in the food and feed industry.

Keywords: Food security, edible insects, alternative protein, insects as food and feed, circular economy

CO276

INSECT PROTEIN FOR ANIMAL FEED – PROGRESS AND CHALLENGES

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An expanding global population together with a greater demand for meat, driven by an emerging global middle class, is placing increasing pressure on the production of protein from sustainable sources. Research over the last decade has shown that insects offer great potential to reduce this protein gap through the use of insect derived protein in feed for monogastric animals and fish. Research for this purpose has focussed on two fly species, *Hermetia illucens* (black soldier fly) and *Musca domestica* (house fly). In addition to the protein, there is also interest in the lipids, chitin and other novel compounds that can be derived from insects. These insects can utilise a wide range of organic substrates for reproduction, which offers the potential for low environmental and economic production costs. Significant progress has been made in this area with a greater understanding of the biology and behaviour of these insect species together with animal feeding trials to demonstrate the use of insect-derived protein on digestibility and animal growth. A review of the progress made together with some of the challenges remaining will be presented.

Keywords: Insect protein, animal feed, *Hermetia illucens*, *Musca domestica*

Session 10.
Medical and Veterinary Entomology

Epidemiology and Management of Insect Vectors

CO277

ANOPHELES MOSQUITOES OF THE MACULIPENNIS COMPLEX IN EMILIA-ROMAGNA AND LOMBARDY (NORTHERN ITALY)

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Sabrina Canziani, IZSLER, Italy
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After the Malaria eradication in Italy, the interest in characterization the Anopheline fauna has progressively decreased, and nowadays, the knowledge of the distribution of these mosquitoes is largely fragmentary. If mosquito vectors are present, autochthonous Malaria cases are still possible the arrival of infected people, as demonstrated by the recent outbreak in Greece or the autochthonous cases in Puglia during 2017. The definition of Anopheline fauna is hampered by the presence of complex of sibling species difficult to distinguish on a morphological basis. The Maculipennis complex is one of these groups, which includes primary Malaria vectors, and other species considered secondary or irrelevant vectors. Seven species of the complex are reported in Italy: *Anopheles labranchiae*, *An. sacharovi*, *An. atroparvus*, *An. maculipennis ss*, *An. melanoon*, *An. messeae*, *An. subalpinus*. The aim of this study is to define Maculipennis complex species present in studied area. Anopheles mosquitoes were directly collected (mainly by manual aspiration in resting sites) or retrieved from the entomological surveillance programs (particularly WNV surveillance plans). To preserve the body, DNA was extracted from two legs and submitted to ITS2 and COI PCRs and the obtained products were sequenced for molecular identification. Morphometry was applied to wings, in attempt to discern the different species preserving mosquitoes, which can thus be addressed to molecular analyses after species identification. More than 10,000 Anopheles mosquitoes were sampled, mainly in 2017. 420 specimens were identified by biomolecular analysis, of which 353 from Emilia-Romagna and 67 from Lombardy. The most abundant species was *An. messeae* (333 specimens), followed by *An. maculipennis ss* (78 specimens), more abundant in the western part of the surveyed area. Moreover 9 specimens of *An. atroparvus*, a good vector of Malaria, were identified. These preliminary results demonstrate that Anopheles mosquitoes, potentially able to transmit Malaria, are still present in Northern Italy.

Keywords: Maculipennis complex, *An. messeae*, *An. maculipennis ss*, *An. atroparvus*, Malaria

CO278

CLIMATE CHANGE AND VECTOR-BORNE DISEASES IN EUROPE

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Zika, dengue, chikungunya, malaria, Lyme disease, bluetongue, Shmallenberg are vector-borne diseases (VBD) with huge impacts on societies and they are now omnipresent in the news. These diseases are transmitted by exothermic arthropod vectors such as mosquitoes, ticks and midges which are extremely sensitive to external environmental conditions. Rainfall is an important factor as it provides breeding sites for larvae. Temperature impacts a broad range of factors such as vector development, its survival, vector biting rates and the time required for the pathogen to develop inside the arthropod vector (so called extrinsic incubation period). Consequently, future climate change is expected to greatly impact the distribution and severity of these vector-borne diseases. Different mathematical models can be used to model the dynamics of vector-borne diseases. We employ various mechanistic models for which epidemiological parameters dynamically rely on climatic variables. This presentation will present recent modelling advances about climate change impacts on animal and human vector-borne diseases, with a focus on recently reported trends in Europe. We will discuss the risk of arbovirus transmission posed by the Asian tiger mosquito *Ae. albopictus* and the risk posed by bluetongue virus to animal health in Europe. To conclude, we will present recent modelling results showing that the climate phenomenon El Niño fuelled the Zika outbreak that plagued Latin America in 2015-16.

Keywords: Climate change, epidemiological modelling

CO279

EPIDEMIOLOGY AND MANAGEMENT OF CULICOIDES AND CULICOIDES-BORNE DISEASES IN EUROPE

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Arboviruses transmitted by Culicoides biting midges have undergone an unparalleled emergence in Europe during the past two decades, involving major changes in their epidemiology. In this talk I will draw parallels with the emergence of other vector-borne diseases in Europe to examine differences in the potential for sustained transmission of arbovirus in this region. This will include examining our response to veterinary *versus* medical pathogens and how the requirements of policy shape the research we conduct. The aim is to highlight where surveillance in particular can be harmonised across vector groups and to make schemes more effective in providing the data we require. I will examine the methods used to define both spatial and temporal incidence and some of the strengths and weaknesses of datasets produced from these schemes. Then I will discuss how future advances in technology may change the ways in which we carry out surveillance, summarising emerging techniques in this area. Finally I will discuss areas of weakness in our current systems for responding to arbovirus incursions with reference to our experience with bluetongue and Schmallenberg viruses.

Keywords: Biting midges, arbovirus, surveillance, emergence

CO280

MODELLING VECTOR DYNAMIC AND ARBOVIRUS OUTBREAKS IN EUROPE

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The colonization by mosquito species such as *Aedes albopictus* of Europe represents an alarming public health threat due to their potential role as competent vectors for arboviruses. Moreover, the expanding geographic range of mosquito-borne diseases such as Dengue and Chikungunya and the re-occurrence of significant outbreaks in endemic countries have increased the risk of infected travellers reaching Europe. The combination of these factors has already had an impact. Indeed, Southern Europe experienced in the last decade events of autochthonous transmission of Dengue in France and Croatia and two Chikungunya outbreaks in Italy. Mathematical and statistical models can improve our knowledge on vector and outbreak dynamics, which in turn could help reduce the public health burden represented by mosquito-borne diseases. In fact, understanding the vector population dynamic is essential to identify under which conditions the transmission risk is higher. Moreover, estimates of the vector population dynamics and of the likelihood and impact of arbovirus transmission could help the planning of preventive and reactive interventions. Using the recent (2017) Italian outbreak of Chikungunya, the application of mathematical and statistical models will be showcased to illustrate the quantitative estimation of vector dynamics and transmission risk and their potential to understand actual outbreak dynamics and to inform control interventions.

Keywords: Outbreaks, vector dynamics, arboviruses, mathematical model, Aedes species

CO281

CURRENT DISTRIBUTION OF THE INVASIVE MOSQUITO *AEDES KOREICUS* (DIPTERA; CULICIDAE) IN ITALY

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Gioia Capelli, Istituto Zooprofilattico Sperimentale delle Venezie, Italy

Mosquitoes belonging to genus *Aedes* are often recorded out of their native places as invasive species. In addition, these species are proven or potential vectors of important arboviruses and are a threat for human and animal health. Several invasive Aedes species are now established in Europe and Italy is one of the most infested European countries. At present, three invasive species occur in Italy: *Aedes albopictus*, *Ae. japonicus* and *Ae. koreicus*. The latter, was first found in North-Eastern Italy in 2011. The constant monitoring of this species shows an increasing spreading trend. Aedes koreicus develops in artificial containers, sometimes sharing the breeding sites with the other invasive mosquito species and is adapted to tolerate the cold winter temperature. For this reason, it is present in mountainous and hilly areas where *Ae. albopictus* is present in low density or absent. Starting from the area of the first report, it has now spread over five Italian Regions and 123 municipalities, from West to East colonizing wide areas of Northern Italy. According to these records, northern Italy has a high probability to be invaded by *Ae. koreicus* in the next decade. The North-East Italy in particular, confirms to be one of the areas with the most frequent experience of invasive mosquito introduction in Europe. This is likely a consequence of the intensive trade of goods. The establishment of invasive mosquito species complicates the current surveillance system and requires well trained personnel for identification. A new competent vectors of pathogens may represent a challenge for the Health System.

Keywords: *Aedes koreicus*, North Italy, invasive mosquitoes

CO282

MANAGEMENT OF INSECTICIDE RESISTANCE IN MOSQUITO VECTORS OF HUMAN DISEASE

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Malaria prevention is reliant on insecticides, used to treat bednets or for spraying houses. Coverage with these mosquito control measures has been dramatically scaled up across Africa in the 21st century and this has contributed to the reductions in malaria burden witnessed by many countries. However intense selection pressure with pyrethroid insecticides, the only class currently used to treat bednets, has resulted in high levels of resistance in African malaria vectors. Whilst the impact of resistance on indoor residual spraying is clear, and has led to changes in insecticide use, the impact of resistance on bednets is harder to quantify. The majority of studies attempting to address this have assessed the level of personal protection provided to a bednet user and concluded that bednets are still protective against malaria, even in areas where the mosquitoes are resistant to malaria. However the success of bednets in controlling mosquito populations relies on the additional community level protection afforded by the killing action of the insecticide, which reduces the longevity of the mosquito population and provides protection to both net users and non users. This community effect will be rapidly eroded by pyrethroid resistance. Data from laboratory and field studies, plus outputs from modeling studies, will be presented to outline the current status of our understanding of the impact of insecticide resistance. Alternative approaches to manage resistance, including critical knowledge gaps, which are impeding the implementation of effective resistance management strategies, will also be discussed.

Keywords: Malaria, bednet, pyrethroid, insecticide, anopheles

CO283

MANAGEMENT OF TICK INFESTATION IN WILD ANIMALS

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Tick borne diseases represent an increasing public health threat worldwide. The tick *Ixodes ricinus* is a competent vector for a number of zoonotic pathogens which include viral infections such as tick borne encephalitis and bacterial infection such as *Borrelia burgdorferi sensu lato* and *Anaplasma phagocytophilum* among others. Many wildlife species act as competent reservoirs for these pathogens although their reservoir capacity varies accordingly to several factors. Control of tick borne diseases in wildlife is challenging and several attempts have been carried out in the last decade without significant success in term of costs/effectiveness. However, surveillance of tick borne diseases in wildlife can provide irreplaceable information on changes in hazard and therefore provide early warning signals to be used by public health authorities to prevent infection and properly warn the population at risk

Keywords: *Ixodes ricinus*, emerging tick borne diseases, management, control, surveillance

CO284

RECENT ADVANCES IN THE USE OF ENTOMOPATHOGENIC FUNGI FOR THE CONTROL OF MOSQUITOES

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The use of entomopathogenic fungi to control the larval and adult stages of vector mosquitoes is gaining momentum. From the realization that fungi which are not found naturally infecting mosquitoes, could be potential control agents, research has now concentrated on species normally used against agricultural pests such as *Beauveria bassiana* and *Metarhizium anisopliae*. One of the main advantages of using these fungi is the ease of mass production, especially when compared to species which are natural pathogens of mosquitoes. Certain challenges still need to be addressed, such as the development of efficient delivery systems and increasing persistence in the field. Our research on the control of *Aedes aegypti* larvae is now focusing on the use of *Metarhizium blastospores*, which are highly virulent, killing 100% of the larvae in 24-48h. However, blastospore virulence declines rapidly with a half-life of approximately 12 days. We are currently working on encapsulation to improve persistence under field conditions. A variety of systems have been tested to deliver fungal inoculum to adult mosquitoes. The basis of many of these systems is to impregnate fungi on surfaces which mosquitoes land and thus become infected. Therefore, it is important that mosquitoes are attracted to land on these surfaces. Our research has concentrated on the use of fungus impregnated black cotton cloths. These cloths are deployed by hanging them inside plastic bottles with one side cut away to allow easy access of mosquitoes to the cloths. Mosquitoes are naturally attracted to land on the cloths and significant reductions in survival are obtained when tested in rooms simulating human residences. In order to increase the landing frequency of mosquitoes on the cloths, the addition of a synthetic attractant was tested. AtrAedes® together with *M. anisopliae* significantly reduced survival rates and this system will now be tested in human residences.

Keywords: Insect, vector, fungus. virulence, biological control, disease

CO285

ASSESSING THE DIVERSITY OF SPIDERS USING DNA BARCODING

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In the present study we identified spiders of different families morphologically and then authenticity of morphological identification was confirmed by using DNA barcoding. The spiders were collected and preserved in 95% alcohol. The DNA was isolated from left leg of spider following the protocol of Thermo Scientific GeneJET Genomic DNA Purification kit. Standard barcode region of *COI* gene of 64 samples was amplified. The sequences of 658 base pairs were recovered from 62 samples, representing 7 families, 20 genera and 27 species. Araneidae was the most dominant family in followed by Salticidae, Oxyopidae, Clubionidae, Tetragnathida, Thomisidae, Mitergidae and Lycosidae. The interspecific value of divergence was more than the intraspecific value of divergence for all seven families which described a clear barcode gap. No overlap was recorded in intra-specific and inter-specific divergence values. Furthermore, distance to NN was higher than the maximum intra-specific value for all species. We concluded that spiders can be DNA barcoding is a reliable able tool for identifying spiders.

Keywords: Spiders, DNA barcoding, *COI* gene, morphological identification, cryptic species.

Vector and Pathogen Biology

CO286

MULTI-SCALE OVIPOSITION SITE SELECTION IN TWO MOSQUITO SPECIES

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In organisms characterized by complex life cycles, undergoing ontogenetic niche shifts, habitat selection is hierarchical processes occurring at multiple spatial scales. In mosquitoes, the immature stages are confined to aquatic habitats, while adults are free ranging within the surrounding terrestrial landscape. Hence, females searching for an oviposition site should consider the characteristics of the aquatic habitat and terrestrial landscape in which it is embedded. We conducted a field experiment testing for multi-scale oviposition site selection in two mosquito species. Artificial pools were placed in two adjacent landscapes, olive plantations and citrus orchard, differing in their blooming period and nectar availability. Within each landscape, pools were organized in three pairs: 1) caged-fish was present in both pools, 2) caged-fish was present only in one pool, and 3) fish was absent from both pools. In the first experimental run, most of the egg rafts were identified as *Culiseta longiareolata*, and they were mainly found in pools located in the citrus orchard. *Culiseta longiareolata* females also laid most of the eggs during the second experimental run, but their preference to the citrus orchard diminished. During the third run, most of the eggs were laid by *Culex pipiens* females, yet the majority of them were found in pools within the olive plantation. The two mosquito species also displayed different patterns of oviposition site selection, with *C. pipiens* laying its eggs almost exclusively in fish-free pools, and *C. longiareolata* also accepting pools containing caged-fish. *Culex pipiens* was much more selective at both spatial scales, probably due to lower movement cost and stronger ability to gather environmental information. A trade-off among different gonotrophic cycles, combined with time limitation associated with a shorter breeding season may have lowered *C. longiareolata* selectivity. These differential oviposition patterns can strongly affect the population and community dynamics of both species.

Keywords: Complex life cycle, Incomplete information, Mosquitoes, Multi-scale habitat selection, Population persistence, Predation risk, Reproductive trade-offs, Spatial contagion

CO287

SALIVARY MICRORNAS FROM ANOPHELINE MOSQUITOES: ADDITIONAL PLAYERS IN VECTOR-HOST-PATHOGEN INTERACTIONS?

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Saliva of hematophagous arthropods carries antihemostatic, antiinflammatory and immunomodulatory activities playing crucial roles in blood feeding and vector-host-pathogen interactions. In the last decades transcriptomic and proteomic studies clarified the complexity of mosquito saliva shedding light on several novel physiological functions and mechanisms of action of salivary proteins. However, animal body fluids, including saliva, are known to comprise different types of non-coding RNAs and microRNAs have been recently described in the saliva of arthropod vectors as Aedes mosquitoes and Ixodes ticks. To get insights into the salivary miRNA repertoires of anopheline mosquitoes we performed a small RNAseq analysis on saliva, salivary glands, adult males and adult females of *Anopheles coluzzii* (formerly known as *A. gambiae* M molecular form). Overall, we obtained evidence of expression for at least 214 miRNAs, with 36 representing putative novel anopheline miRNAs, and identified 30 female salivary gland-enriched and 68 sex-biased miRNAs. Saliva included at least 77 miRNAs and, noteworthy, part of them were asymmetrically distributed between salivary glands and saliva, suggesting that selected miRNAs may be preferentially directed toward the secretory pathway. Intriguingly, 10 out of the 20 most abundant salivary miRNAs were essentially identical to human miRNAs targeting genes involved in host immune and inflammatory responses. This study expands the miRNA repertoires of the African malaria vectors *A. coluzzii* and *A. gambiae* providing the first evidence that specific miRNAs, perhaps under the evolutionary pressure imposed by blood feeding, may be preferentially conveyed to mosquito saliva. A relevant implication, with possible consequences for pathogen transmission, is that manipulation of host responses by mosquito saliva may take place not only through the biochemical and pharmacological properties of salivary proteins but also by miRNA-mediated post-transcriptional regulation of host gene expression.

CO288

HOW TO OBTAIN CULICOIDES ABUNDANCE DATA AT A NATIONAL SCALE WITH ONE COLLECTION SEASON IN MOROCCO

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For vector-borne diseases, producing abundance maps is a necessary step for risk mapping, which is an important tool to help focusing surveillance actions. Such as mapping requires sampling vector insects at a national/regional scale, which is quite difficult due to important heterogeneity in spatial distribution and in temporal dynamics. In Morocco, Culicoides biting midges were responsible for African horse sickness (AHS) outbreaks in the 1960s and in the late 1980s. They are also involved in the enzootic transmission of bluetongue (BT) virus since 2004. We aimed to produce first abundance maps for most abundant Culicoides species, and then risk maps for BTV and AHSV, across Morocco. In 2016, we compared species diversity in ruminant farms and in horse holding to determine if abundance maps derived from sampling in ruminant farms could be used to generate risk maps for both AHS and BT viruses. In 2017, we carried out a cross-sectional survey at the national scale. We used a stratified sampling strategy using eco-agronomic zones, with which we hypothesized homogeneity of Culicoides diversity and dynamics. We used historical collection time series to determine periods of abundance peak according to climatic zonation. This strategy allowed us to plan a national sampling of Culicoides abundance. In 2016, we found the same species in cattle and horse holdings. In addition, Culicoides paolae and Culicoides scoticus were recorded for the first time in Morocco. In 2017, collection campaign was conducted twice a year (from April to June and from September to October): 144 farms were trapped during this period. At each visit, Culicoides populations were sampled for 48 consecutive hours using a light trap. A total we obtained 262 samples. Samples were currently under identification process. The next step will be to determine ecological drivers of abundance and distribution to provide accurate Culicoides mapping across Morocco.

Keywords: Culicoides, Morocco, Bluetongue, Abundance

CO289

DEMOGRAPHIC HISTORY OF *AEDES ALBOPICTUS* POPULATIONS AND ARBOVIRUS TRANSMISSION

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Using population genetics analyses and Approximate Bayesian Computation (ABC) approaches together with historical observations we are inferring the demographic history of *Ae. albopictus* populations from representative countries in the Southeast Asian native range and in the recent and more recently colonized areas around the world. We found that the diffusion of this mosquito has not occurred through a progressive expansion away from the native Southeast Asia. A recent and chaotic human-mediated propagule distribution allowed adventive populations to be established in the Indian Ocean, Mediterranean Basin, North /South America and Africa. As a consequence, we have detected a great amount of intra-population variability, and it is likely that this variability may extend to the genetic mechanisms controlling vector competence. Indeed, we found that differences in vector competence are observed according to the demographic history. Thus, in the context of the invasion process of this mosquito, it is possible that both population ancestry and admixture contribute to create the conditions for the efficient transmission of arboviruses and for outbreak establishment.

Keywords: *Aedes albopictus*; demographic history, arboviruses, vector competence

CO290

EFFECT OF MOSQUITO AGE, EXPERIENCE AND INFECTION STATUS ON DEET EFFICACY IN THE MALARIA MOSQUITO *ANOPHELES GAMBIAE S.S.*

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Mosquito-borne diseases are major public health concerns, and their control is threatened by the spread of insecticide resistance in vector populations. In this context, the use of repellents is an alternative strategy to limit mosquito-host interactions. However, mosquito physiological condition is thought to affect their efficacy. Particularly, experience, age and infection status are of great epidemiological interest, and have been rarely investigated. Yet, as mosquitoes get infected during blood feeding, infectious mosquitoes are expected to have experienced at least one contact with a repellent in areas of high repellents coverage. Also, the extrinsic incubation period of some pathogens is relatively long, which means that old mosquitoes are the most susceptible to be infectious. Finally, mosquito infection can influence olfactory perception or behavior. In this context, the efficacy of the DEET repellent against experienced, old, and infectious malaria mosquitoes *Anopheles gambiae ss* females was evaluated. Three independent experiments were performed. First, we evaluated whether a first blood meal successfully obtained upon a DEET–treated net would influence the success at taking a second blood meal in spite of DEET. Then, we compared inhibition by DEET between mosquitoes aged of 4, 11 and 18 days. Finally, we evaluated DEET inhibition in uninfected mosquitoes and in mosquitoes infected at different developmental stages of *P. falciparum*. Results reveal complex interactions between mosquito physiological condition and repellents efficacy, with for instance no effect of a prior exposure to DEET on its efficacy at the second exposure, but a strong increase in DEET efficacy with mosquito aging. Our results give insights to understand the mode of action of the most used mosquito repellent. They also could have significant epidemiological consequences for vector control and may help public health policies in designing protection tools that specifically target mosquitoes that are the most susceptible to transmit pathogens.

Keywords: DEET, *An. gambiae s.s.*, experience, age, infection

CO291

LARVAL SUPERIORITY OF *AEDES ALBOPICTUS* TO *CULEX PIPIENS* OR VICE VERSA?

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The Asian tiger mosquito *Aedes albopictus* is an important vector of various human and veterinary pathogens, which currently establish first populations in Northern Europe (Germany). Here *A. albopictus* will frequently encounter the common house mosquito *Culex pipiens*. While competitive superiority of *Ae. albopictus* to *Cx. pipiens* (s.l.) has been described elsewhere, it has not been assessed in cold-temperate environments. The present study aims to determine competitive relationships between two mosquitoes under physicochemical and microclimatic conditions typical for container habitats in Germany. In a replacement series experiment, the larval and pupal responses of *Ae. albopictus* and *Cx. pipiens* (s.l.) (mortality, development time, growth) to interspecific interaction (five larval ratios) at (sub-)optimal temperatures (15, 20 and 25°C) and differing food supply (3 and 6 mg animal-based food larva-1) were investigated. In addition to physicochemical measurements of the test media, natural physicochemical conditions were determined for comparative analyses in mosquito breeding sites across the Rhine-Main metropolitan region of Germany. Under the physicochemical and microclimatic conditions similar to the breeding sites of the Rhine-Main region, competitive superiority of *Cx. pipiens* (s.l.) to *Ae. albopictus* in terms of larval survival was more frequently observed than balanced coexistence. Food regime and multifactorial interactions, but not temperature alone, were controlling factors for interspecific competition. Larval food regime and the larval ratio of *Ae. albopictus* influenced the physicochemistry and algal growth at 15°C, with increased *Ae. albopictus* mortality linked to a decreasing number of Scenedesmus, Oocystis and Anabaena algae. Under the present environmental conditions, the spread of *Ae. albopictus* from isolated foci in Germany may generally be slowed by biotic interactions with the ubiquitous *Cx. pipiens* (s.l.) (and potentially other container-breeding mosquito species) and by limnic microalgae in microhabitats with high resource levels. Detailed knowledge of the context dependency in temperate mosquito ecology, and interrelations of physicochemistry and phycology may help to achieve a better understanding of the upcoming *Ae. albopictus* colonization processes in central and northern Europe.

CO292

A CRUCIAL ROLE FOR GUT MICROFLORA IN THE LIFE HISTORY OF THE MAJOR MALARIA VECTOR *ANOPHELES ARABIENSIS* (DIPTERA: CULICIDAE)

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The gut microbiome is a crucial determinant of a variety of life history traits in both vertebrates and invertebrates. In this study laboratory strains of *Anopheles arabiensis* were used to determine the role of gut microflora in life history traits of epidemiological importance. The SENN strain is an insecticide susceptible strain from Sennar, Sudan and the SENNN-DDT strain was selected from this strain and displays multiple resistance. Four antibiotics were used to preferentially affect the gut microflora; gentamycin (broad-spectrum, bactericidal), erythromycin (broad spectrum, bacteriostatic), vancomycin (gram-positive bactericidal) and streptomycin (gram-negative bactericidal). The antibiotics were supplied via sucrose supplementation to determine the effects on adult longevity and insecticide sensitivity. Antibiotics supplemented via blood was used supplied to determine the effect on insecticide sensitivity. For the SENN strain, erythromycin reduced longevity, but none of the bactericidal antibiotics affected longevity. Bactericidal antibiotics had a more marked effect on SENN-DDT, with gentamicin reducing longevity in both sexes and vancomycin reducing

male longevity. Vancomycin supplementation via sugar had the greatest effect on the resistance phenotype, increasing deltamethrin and malathion-induced mortality in male and female SENN DDT, although all antibiotics increased malathion and deltamethrin resistance in either males or females. Conversely, supplying the adults with *Escherichia coli* or *Streptococcus pyogenes* decreased malathion and deltamethrin induced mortality. A similar pattern was observed when antibiotics were supplemented with vancomycin increasing both malathion and deltamethrin induced mortality, and negating the protective effects of a blood meal. Matrix-assisted laser desorption time of flight mass spectroscopy analysis of the bacterial content of the gut shows a significant decrease in the bacterial diversity of the resistant strain. These results suggest a marked difference in the biodiversity of gut bacteria in insecticide resistant and susceptible An. arabiensis, as well as a crucial role for gut bacteria, specifically gram-positive bacteria in the resistance phenotype.

Keywords: Anopheles, microflora, insecticide resistance, longevity

CO293

LEISHMANIA DEVELOPMENT IN PHLEBOTOMINE SAND FLIES (DIPTERA: PSYCHODIDAE)

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Natural sand fly vectors are very susceptible to Leishmania and 1-2 parasites is enough to initiate mature infections. Blood taken by sand fly females (usually from 0,6 to 0.9 microliters) is directed to the abdominal midgut and surrounded by peritrophic matrix (PM). Aflagellated amastigotes ingested along with a bloodmeal transform to flagellated promastigote forms which are protected against proteolytic damage by surface phosphoglycans. The PM represents an important mechanical barrier, promastigotes escape to ectoperitrophic space only after PM disintegrates. Some sand fly species, like *P. papatasi* and *P. sergenti*, display strict specificity for Leishmania species they transmit and are called, “specific” or “restrictive vectors”. On the other hand, the majority of Phlebotomus and Lutzomyia species support late stage development of multiple Leishmania species and are called “permissive vectors”. There is also a third category, sand flies refractory to all Leishmania tested. The midgut binding mechanism differs between specific and permissive vectors. In *P. papatasi* the attachment to midgut microvilli is controlled by galectin which serves as a receptor for terminal galactose present on *L. major* and *L. turanica* lipophosphoglycan and sems to involve also the flagellar protein FLAG1/SMP1. In permissive sand flies various Leishmania attach due to unspecific binding to mucin-like molecules in sand fly midgut. Once established in the midgut, parasites replicate vigorously and then migrate anteriorly to thoracic midgut. They accumulate in large numbers, produce promastigote secretory gel which physically obstructs the gut. This, together with damaged stomodeal valve, facilitates reflux of parasites from the midgut. The so called “blocked sand flies” have problem to take a bloodmeal, bite repeatedly, increasing the chance of Leishmania transmission.

Keywords: Leishmania. Phlebotomus. Lutzomyia. Vector-parasite interaction.

EU Networks and Infrastructures in Vector Research

CO294

EUROPEAN SOCIETY FOR VECTOR ECOLOGY: THE HISTORY, THE PEOPLE, THE SCIENCE

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The European Society for Vector Ecology (E-SOVE) is the European branch of the American Society SOVE, a nonprofit organization founded in California in 1968. The society is committed to share knowledge and create a large network among scientists on thematics concerning the ecology and control of arthropods of medical and veterinary importance. More than 554 members from 35 countries work together under the umbrella of SOVE to promote global public health through research and vector management. Members represent an amalgamation of diverse professionals in research, medicine, operational and extension personnel from all over the world. Among the main tasks of the E-SOVE are the biennial conferences in Europe. The Society publishes the Journal of Vector Ecology biannually containing research and operational papers covering various topics in vector ecology, biology, genomics and control. SOVE also publishes a newsletter every quarter which is available online to all the SOVE members.

CO295

THE WIN INITIATIVE: A GLOBAL NETWORK TO COMBAT INSECTICIDE RESISTANCE IN ARBOVIRUS VECTORS

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Arbovirus transmitted by Aedes mosquitoes, such as dengue, Zika, chikungunya and yellow fever are re-emerging all over the world. Vector control plays a key role in disease prevention but the use of the same chemicals for decades together with the dissemination of vectors resulted in the global spread of insecticide resistance. A coordinated approach is imperative to detect and manage insecticide resistance. Initiated with the support of the WHO Special Programme for Research and Training in Tropical Diseases (WHO/TDR) and the Department of Neglected Tropical Diseases (WHO/NTDs), the Worldwide Insecticide resistance Network, WIN (http://win-network.ird.fr/) brings together several internationally recognized institutions in vector research to track and combat insecticide resistance in mosquito vectors of arboviruses at a global scale. The missions of WIN are i) to establish a global resistance surveillance system for arbovirus vectors, ii) to identify research priorities and fill knowledge gaps on insecticide resistance, and iii) to assist WHO and national authorities in decision-making on insecticide resistance management and deployment of resistance-breaking tools. Since its creation in 2016, the WIN has organized an international conference on vector resistance in Brazil and produced in-deep reviews to support the development of a global plan for insecticide resistance management in arbovirus vectors. The WIN is now becoming an independent membership organization open to all vector control actors including academia, international organizations, public health agencies, NGOs and industries willing to synergize their efforts for combating insecticide resistance in arbovirus vectors worldwide.

Keywords: Insecticide Resistance, International Network, Arbovirus vectors

CO296

AEDES INVASIVE MOSQUITOES COST ACTION

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COST (CO-operation in Science and Technology) is a pan-European intergovernmental framework dedicated to networking activities for European researchers, engineers and scholars to jointly develop their ideas and new initiatives across all scientific disciplines through trans-European coordination of nationally funded research activities. COST funds networking activities (Meetings, Training Schools, Short-Term Scientific Missions and Dissemination Activities), but does not fund research or equipment purchase (http://www.cost.eu/). The AIM-COST Action intends at overcoming the fragmentation in expertise and implementation of surveillance and control of Aedes Invasive Mosquitoes (AIM) among European countries and at innovating the field of AIM research, prevention and control (and more generally of medical/veterinary entomology and epidemiology of AIM-borne diseases). This will be achieved by developing synergies between scientists, decision-makers, productive sector and civil society necessary to harmonise and rationalise sustainable approaches - both conventional and novel – for the surveillance, control, analysis and dissemination across Europe and beyond. The AIM-COST kick-off meeting on September 6th will mark the official start date of the 4-year ACTION and will see the participation of the Management Committee meeting including to two members from each of the 22 COST Countries, 4 Neighbouring Countries and 2 International Partner. Individuals whose primary affiliation is with an institution located in a COST Full and/or a COST Cooperating Member can join AIM-COST Action as an Action Participant once their respective country has accepted AIM-COST Memorandum of Understanding. The present AIM-COST ACTION network will welcome new participants interested in contributing to AIM-COST activities!

CO297

THE GNATWORK: A RESEARCH NETWORK IN THE STUDY OF BITING MIDGES, SANDFLIES AND BLACKFLIES

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The Gnatwork is a Global Challenges Research Fund (GCRF) funded network, aiming to create and maintain a community of researchers studying biting midge, sandfly and blackfly biology. Although highly divergent in lifecycle, ecology and pathogens transmitted, these biting flies are all major vectors of pathogens affecting humans, livestock and wildlife, and share the similarity of extremely small adult body size. This trait imposes a significant constraint on almost every aspect of research, ranging from the production of laboratory colonies, to studies of their behaviour in the field. The amount of funding available to study these flies also changes over time, making it difficult to retain expertise, particularly in countries where medical and veterinary priorities change rapidly. By enhancing links between researchers, we aim to create a network of scientists around the world working across these three neglected vector groups. As part of the Gnatwork, we will be organising pump-prime funding calls to support up to £600,000 of small-scale collaborative studies, with links to countries receiving official development assistance. We will also host training workshops, in Brazil and Bangladesh, to promote the development of transferable research skills. Overall, we aim to make significant progress in transferring techniques across all three vector groups, developing new ones and building a more resilient research base for biting midges, sandflies and blackflies.

Keywords: Biting midge, blackfly, sandfly, network, community, funding

CO298

MALARIAGEN: BUILDING A DATA-SHARING NETWORK AND TECHNOLOGY PLATFORM FOR LARGE-SCALE GENOMIC EPIDEMIOLOGY AND SURVEILLANCE OF MALARIA VECTOR POPULATIONS

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We describe the development of an open, collaborative, data-sharing network and technology platform, leveraging genome sequencing to undertake coordinated surveillance of malaria vector populations. In Africa the network has two overarching goals. The first goal is to realise the use of genome sequencing as an operational tool for insecticide resistance management (IRM), providing real-time information on the emergence and spread of insecticide resistance outbreaks, and helping to build an evidence base for optimal deployment of vector control products in different settings and regions. The second goal is to accelerate the development of new vector control tools, particularly new insecticides, synergists, and gene drive systems, and prepare for their deployment, by using inferences from genetic data to provide a baseline for current vector population sizes, composition, temporal dynamics and migration behaviours. In Asia the goal is to survey the full taxonomic diversity of malaria vector species, and to identify and characterise the species and populations that are primarily responsible for malaria transmission in different geographical locations, especially transmission of parasites resistant to antimalarial drugs. Building on insights, technologies and best practices developed in the *Anopheles gambiae* 1000 Genomes Project, the Vector Observatory has been operating in a pilot phase since 2017, and has been establishing lab, data processing and analytical pipelines to accommodate whole genome sequencing of 10,000 mosquito specimens per year, as well as developing new sequencing technologies that can scale to larger sample sizes and be deployed to regional facilities. We give an overview of the underlying technologies and infrastructure, current projects and partnerships within the network, the datasets generated so far, the model for participation in the network, and the roadmap for future data generation and technology development.

Keywords: Anopheles, malaria, next-generation sequencing, disease surveillance

CO299

PARTNERSHIP FOR INCREASING THE IMPACT OF VECTOR CONTROL

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Many of our current tools to control vector borne diseases are inadequate in twenty first century urban environments or failing due to insecticide resistance, and the evidence base for newer interventions remains weak. Selecting the most cost effective, affordable and acceptable tools to reduce the burden of vector borne disease requires an integrated approach involving vector biologists, economists and anthropologists, working in partnership with national ministries of health, planning and finance. In reality, most disease endemic countries lack the capacity to develop evidence based strategies to control these diseases. There is a particularly acute shortage of vector biologists but also an urgent need to attract leading scientists from other disciplines to tackle the persistent burden of vector-borne disease. The newly established Partnership to Increase the Impact of Vector Control (PIIVeC), funded by the UK Global Challenge Research Fund, brings together leading research institutes and national disease control programmes from three African countries, Burkina Faso, Cameroon and Malawi, to develop evidence based solutions for integrated vector control. We have appointed 12 African research fellows who will be supported by a multi disciplinary mentorship team to generate new knowledge and tools.

Technical vector control advisory groups have been established in each country to improve integration between separate disease control programmes and to ensure our research is responsive to country priorities. Working with experts in research uptake PIIVeC aims to increase both the supply and demand evidence at national level, leading to informed and sustainable approaches to tackle the burden of vector borne disease in Africa and to increase countries resilience for responding to outbreaks

Keywords: Capacity strengthening, network, partnership, vector borne disease

CO300

THE INFRAVEC2 INFRASTRUCTURE PROJECT: PROVIDING VECTOR RESEARCHERS WITH NO-COST RESOURCES, SERVICES AND FACILITY ACCESS

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The Infravec2 infrastructure project provides insect vector resources and facility access to researchers worldwide at no cost. The project is funded by the European Commission Horizon 2020 Research Infrastructure Program (INFRAIA). The 24 Infravec2 partners operate major European biosecure insectaries for experimental infection and containment of insect vectors and other key insect vector technology platforms including front-line field sites in Africa, the Pacific, and the Americas. Researchers can shop online and request resources from an extensive product catalog (www.infravec2.eu). Infravec2 is also developing innovative new research tools, providing training courses, and is networking the community with activities such as common experimental standards and protocols to obtain reproducible vector infection results across different facilities. Infravec2 is a source of EU research support for vector researchers. The Infravec2 goal is to accelerate European innovation in basic and translational insect vector biology, and to consolidate a high-quality insect vector infrastructure with long-term perspectives for improving global public health.

Keywords: Vector products, no-cost, online shop

Dermanyssus Gallinae: An Endless Threat to the Industry and Human Health

CO301

ATTRACTIVENESS OF VOLATILE ORGANIC COMPOUNDS ON *DERMANYSSUS GALLINAE* (MESOSTIGMATA: DERMANYSSIDAE)

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The Poultry Red Mite (PRM), *Dermanyssus gallinae*, is an obligate blood-sucking ectoparasite of economic importance in layer farms. Increasing acaricide resistance and strengthening of environmental policies make PRM control challenging and require alternative control tools. Whilst the general life cycle and habits of PRM are well established, determinants of its interaction with environment keep poorly understood. The role of general host-related stimuli on the behavioral response of the PRM, like CO^2 and temperature, was well investigated. However, the role of volatile organic compounds (VOCs) other than CO^2 found in the chemical landscape constituted by the henhouse environment on PRM behaviour received little attention. The aim of the study was to explore the attractiveness of some of the VOCs present in farm buildings and their interaction with temperature. We evaluated the attractive response of starved PRM female adults to 8 volatiles compounds commonly found in the farm context, either alone or associated in a blend, in two choice test bioassays (one performed in a Y-tube olfactometer, another one in a field-mimicking lab design). The impact of temperature on the attractiveness of VOCs was studied using a heated substrate. Our results showed that a single VOC (ammonia) and one VOC blend were significantly attractant to PRM in our laboratory conditions. The interaction between VOCs and temperature had a significant impact on PRM attraction. Our work brings fundamental information on chemical interactions between PRM and its environment in farm buildings. This study demonstrates the potential of using volatile substances to manipulate the behavior of PRM, combined or not to different stimuli, to elaborate an attract-and-kill control strategy. The authors would like to thank the European Cooperation in Science and Technology (COST Action (FA1404 - COREMI) "Improving current understanding and research for sustainable control of the poultry red mite *Dermanyssus gallinae*").

Keywords: Poultry Red Mite, volatile organic compounds, behaviour, attraction

CO302

FIPRONIL SCANDAL AND THE LESSON LEARNT

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In the last year, the poultry chain has been shaken by the Fipronil scandal, when several farms all over the Europe were found to be positive to that molecule, illegally used to control the infestation by the poultry red mite (PRM) *Dermanyssus gallinae*. This event is leading to some considerations. In the first place, the operators' unawareness of the consequence of such a conduct should be considered. Fipronil is a toxic molecule, harmful for the environment as well as for reared animals and even humans, who may acquire drug residues following consumption of contaminated poultry derived foods. Beyond the impact on health and environment, it is not negligible the negative economic impact on the whole poultry system, which, inescapably, loses its trustworthiness. On the other side, it is clear that the farmers are not the only responsible of this situation. Often, the operators are left alone to face the important problem of *D. gallinae* infestation, whose the majority of audience is even unaware. The drugs designed and authorized for using in the presence of animals are very few, and the situation is complicated by the insurgence of less susceptible mite populations. Therefore, the farmers welcome effective option that can be proposed to them, hoping for a definitive solution to their problem. After these events, it is clear that the approach only based on the use of synthetic or semisynthetic drugs is not the best choice, and this is another clear evidence arisen from the Fipronil scandal. In fact, it should be strongly advisable that the struggle against PRM should be faced by using an integrated strategy, which involves concerted actions of technicians, veterinarians, drug industries and even equipment manufacturers.

The authors would like to thank the European Cooperation in Science and Technology (COST Action (FA1404 - COREMI) "Improving current understanding and research for sustainable control of the poultry red mite Dermanyssus gallinae").

Keywords: *Dermanyssus gallinae*, fipronil; acaricides, mites, poultry

CO303

DERMANYSSUS GALLINAE AND ITS PUBLIC HEALTH CONSEQUENCES

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The poultry red mite (PRM), *Dermanyssus gallinae*, is best known as a threat to the laying hen industry, adversely affecting hen health and welfare through both its feeding behavior and role as disease vector. However, due to its genetic plasticity, PRM attacks wild and synanthropic birds as well and, more importantly, has consequences in non-avian hosts, particularly humans. Poultry workers, farmers, technicians and veterinarians are most at risk for dermanyssosis and the inclusion of PRM as an occupational hazard has been recommended. In the mean time, reports of dermatological complaints have increased in frequency, in several countries, including residential town settings (private homes, hospitals, public offices), typically linked to nearby feral (pigeons, sparrows, etc.) (mostly recently-abandoned) birds' nests. Attacks of *D. gallinae* to humans cause pruritus, skin lesions characterized by small erythematous papules -mostly concentrated on arms, hands, chest, legs - which are often misdiagnosed by medical clinicians. The medical significance of dermanyssosis is exacerbated by the potential of the PRM to carry and transmit zoonotic diseases of both bacterial and viral origin. Additionally, in the poultry farm context, since chemicals are still the dominant means of PRM control, they may have adverse effects on humans, both directly, for workers exposed to chemicals and indirectly through consumption of poultry meat and eggs containing pesticide residues. The recent scandal on the use of an unlicensed product (fipronil) provides clear evidence of such risk. The CoReMi COST Action also focuses on the One Health paradigm, aimed to encourage interactions and exchange information among medical practitioners, environmentalists, veterinarians, academics, industrial researchers, and promoting actions for an increase awareness of *D. gallinae* infestations in humans, mite identification, development of diagnostic tools, advance and use of non-chemicals control measures, in order to limit the consequences of *D. gallinae* and safeguard animal, human and environmental health.

The authors wish to thank the European Cooperation in Science and Technology (COST Action (FA1404 - COREMI)"Improving current understanding and research for sustainable control of the poultry red mite Dermanyssus gallinae").

Keywords: *Dermanyssus gallinae*, mite, birds, humans, One Health

CO304

ALTERNATIVE CONTROL OF *DERMANYSSUS GALLINAE*

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Control methods of poultry red mite (*Dermanyssus gallinae*) historically have relied mainly on synthetic acaricides. However, the repeated long term use of these compounds has resulted in the development of resistant populations of poultry mites, which was confirmed in some countries in Europe, mainly against permethrin and/or carbaryl. The use of essential oils seems to be a promising alternative approach. Although more than 60 plant products have been tested, there is still a lot of problems connected with the use of essential oils such as: persistency of essential oils quality, repeatability of acaricide effect, toxicity for both invertebrates and vertebrates, suitable solvents. The aim of the study was to compare methanol and Tween 85 with distilled water as solvents for essential oils. Toxicity of clove bud, cinnamon bark and lavender against poultry red mites were evaluated. LD50, LD95 and LD99 were lower using Tween 85 in comparison with methanol in clove bud and cinnamon bark preparation. On the other hand mite mortality was much lower using Tween 85 at all levels of dilution (0.25, 0.12, 0.06, 0.03 and 0.015µL/cm^2) with lavender EOs. Essential oils derived from clove buds and cinnamon have been shown to be effective acaricides against the poultry red mites at concentrations 0.5 and 0.25 µL/cm^2, when tested over a 24h period. The average mortality in the negative control was 2%. Biological control by the introduction of predatory mites is another options for controlling poultry red mites. Hypoaspis mites demonstrated the greatest potential as predators of *D. gallinae*.

The authors would like to thank the European Cooperation in Science and Technology (COST Action (FA1404 - COREMI)"Improving current understanding and research for sustainable control of the poultry red mite Dermanyssus gallinae").

Keywords: Poultry red mite, essential oils, predators

CO305

ECONOMIC IMPACT OF *DERMANYSSUS GALLINAE* INFESTATION IN LAYERS FARM: PRELIMINARY DATA

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Dermanyssus gallinae (the Poultry Red mite- PRM) is an ectoparasite, which affects in particular layer farms (enriched cages, aviary or free range systems), causing from mild to severe clinical symptoms (anemia, irritation, feather loss, increased mortality and decreased egg production and quality). The PRM is widely diffuse in European farms and in Italy the estimated prevalence is 83%. There are no specific studies about the economic impact of the PRM in layer farms taking into consideration the costs of treatments, labor, decreased production and mortality. In three different farms (A, B, C), with heavy mite infestations, production and economic data were collected before and after a treatment with fluralaner. In farm A, it has been calculated that *D. gallinae* had a negative impact on egg production of 1.24€/hen and - even with 2 treatments of fluralaner - in a period of 6 months, the Return of Investment (ROI) was 1.09. In farm B, data were collected for 86 days before the molting. An increase of 2.78% in egg production and 2.19gr/egg was recorded. In general, throughout the period of the study, farmers had a return of 83% of the investment. In farm C, data were collected for 148 days. In this farm, after one treatment, the production increased by 2% and the egg weight rose of 1.15g/egg. In this case the farmer had a ROI of 97%. Although these data are preliminary, the economic impact of *D. gallinae* in poultry farms appears to be relevant. A correct approach to control the PRM can provide a prompt positive feedback on production and animal welfare and, as a consequence, on the economic performance of the layer farms.

The Author would like to thank the European Cooperation in Science and Technology (COST Action (FA1404 - COREMI)“Improving current understanding and research for sustainable control of the poultry red mite Dermanyssus gallinae”).

Keywords: *Dermanyssus gallinae*, layer farms, economic impact

CO306

FACTORS AFFECTING POPULATION GROWTH RATE OF THE POULTRY RED MITE *DERMANYSSUS GALLINAE* IN LAYING HEN FACILITIES

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Worldwide one of the most common and significant pest in laying hen facilities is the poultry red mite *Dermanyssus gallinae*. This bloodsucking parasite hides during day time in cracks and crevices in the vicinity of the hen nightly resting place and emerges from there every few days to feed on the laying hen. The mite needs preferably birds blood to fulfil its lifecycle and produce eggs. Large number of mites feeding on the hen result in reduced hen welfare, hen health and reduced egg production, costing the poultry farmer between €0,5 and 1€ per laying hen per year. Control of this pest is difficult and therefore insight in factors affecting the mite population growth contribute to better control the poultry red mite. Therefore monitoring data from 20 flocks in four different laying houses with three different housing systems was analysed using a REML model. This model showed a large variation of the mite population growth rate with variations between flocks and within flocks. 13.2% of this variation could be explained by flock age, house temperature, treatment and hen house. With increasing flock age the mite population growth rate gradually reduces, even without treatment. Treatment effect was age dependent; with an increasing flock age (weeks), the effect of a treatment on population growth rate reduces. With increasing or decreasing temperature the population growth rate respectively increases or decreases. This temperature effect is independent from flock age, treatment or hen house. The effect of hen house on the mite population growth rate was not for all hen houses significantly different from each other. Including a random regression term in the REML model increased the explanation of the variation only by 3,9%. We suggest that farm management may be the main factor explaining the variation in population growth of *Dermanyssus gallinae* in laying hen facilities.

Keywords: Poultry, *Dermanyssus gallinae*, growth rate variation, factors

CO307

THE POTENTIAL FOR VACCINATION TO CONTROL POULTRY RED MITE

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In recent years, several vaccine candidates have been identified in *Dermanyssus gallinae* by employing native protein fractionation and testing and/or by selecting antigens by their inferred orthology with protective antigens from other species. Many of the recombinant *D. gallinae* antigens or native extracts previously tested for vaccine efficacy using an *in vitro* feeding device demonstrated increased *D. gallinae* mortality after mites had fed on blood from immunised hens. However, translation of this efficacy into sustainable control in field tests has had variable results. For example, an extract of *D. gallinae* (termed “SME”) which induced 24% mortality in mites feeding *in vitro* on blood from hens which had been immunised with SME, also resulted in 75% reductions in *D. gallinae* populations when used as a prototype vaccine in a large field trial. In contrast, immunisation of hens with recombinant versions of 3 key immunogenic proteins from SME failed to give any protection in field trials, in spite of results from *in vitro* feeding assays which demonstrated that mites feeding on blood from hens immunised with each of the recombinant proteins were 1.7-2.8 times more likely to die than mites feeding on blood from control hens. To be able to rationally design better, effective vaccines to control poultry red mites we need 3 key resources: 1) a well-annotated genome from which to select and design targets; 2) a reliable means of testing prototype vaccines in small studies *in vivo* and 3) a means of delivering the vaccine to prolong efficacy throughout the egg production cycle. This presentation will address these criteria, identifying gaps and highlighting new knowledge and approaches.

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Keywords: Dermanyssus, poultry red mite, vaccines

CO308

MOLECULAR MARKERS AND THE NEUTRAL GENETIC STRUCTURE OF *DERMANYSSUS GALLINAE* (DE GEER, 1778)

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To understand the epidemiology of the Poultry Red Mite (PRM) *Dermanyssus gallinae* and plan accurate prevention and control means, there is a need to determine how populations of the PRM are connected to each another. Variations amongst pest populations in terms of acaricide resistance, pathogenicity and pathogen vector competence typically result from contributing factors including the level of pre-existing genetic variability in resident population and the frequency with which interbreeding occurs with other populations. Two main reasons for crossbreeding being reduced or null between populations are (1) populations belong to different biological species (including cryptic species, i.e. morphologically indistinguishable species), (2) physical barriers prevent populations to meet with each other. Here we provide a survey of literature on molecular explorations dealing with such questions. All studies used Sanger DNA sequencing (mitochondrial and/or nuclear genes). One study explored the species boundaries and interrelationships within Dermanyssus via a morpho-molecular approach. Four teams studied the intraspecific diversity of PRM in poultry worldwide and discussed results in terms of spread routes. Results revealed that (1) the specific taxon *D. gallinae* encompasses at least two cryptic species,

namely *D. gallinae* s.str. typically infesting poultry farms and *D. gallinae* L1 strictly associated with pigeons, (2) although several different species of Dermanyssus were found in the wild avifauna, *D. gallinae* s. str. was the only species present in poultry farms worldwide, (3) the genetic structure of only *D. gallinae* s.str. was studied to date and results have shown that wild birds were not responsible for PRM spread amongst poultry farms. Instead, mites are spread through farms via industrial routes by anthropogenic vectors. Populations' transfer obviously occurred between farms not only from different European countries, but also worldwide (Europe-Asia, Europe-Brazil). Limits of the current studies and requirements for future studies are discussed.

Authors thank COST Action FA1404 (COREMI).

Keywords: Poultry Red Mite, molecular markers, spread routes, cryptic species

CO309

DERMANYSSUS GALLINAE: THE REASONS FOR A COST ACTION (COREMI)

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Dermanyssus gallinae, also known as the Poultry Red Mite (PRM) is the most debilitating blood-feeding arthropod attacking birds and mammals. It costs circa 230 million euros per year to the European poultry industry and has a prevalence around 80% in many European countries. Since the first paper published 90 years ago on Dermanyssus we have seen over 85 countries publishing on such pest with many papers targeting control methods. As in other pest arthropods, resistance against some pesticides (esp. some of the authorized acaricides) were recorded in some PRM populations. Besides, due to the specific biology of this mite (which spends almost its whole life in the environment at a distance from the host), the efficiency of most sprayed substances is insufficient due to the product not reaching the largest part of the PRM population. Many countries have worked on approaches linked to their national legislation and what products were available and legalised or depending of the research priorities their national funding bodies were interested to support. Such national approach created fragmented data and a lack of potential replication and comparability between countries. Fortunately, since 2014 a new EU initiative from the COST scheme under the COREMI FA1404 Cost Action (www.coremi.eu) has developed a strong network (28 countries and over 300 members) to work together. In recent years the research focus has been towards new control methods including organic/biological approaches, antigen candidates for potential vaccines or understanding the vector capacity, reproduction or detoxification of *Dermanyssus gallinae* and many more. Such collaborative work has created new knowledge on such pest, increasing co-authorship of scientific publications between the COREMI members.

The authors would like to thank the European Cooperation in Science and Technology (COST Action (FA1404 – COREMI)"Improving current understanding and research for sustainable control of the poultry red mite Dermanyssus gallinae").

Keywords: *Dermanyssus gallinae*, COREMI, Poultry pest, One Health

Session 11.
Morphology, Systematics and Phylogeny

Imaging Methods for Scientific Progress in Entomology

CO310

EFFECT OF DENSITY AND HOST DISTRIBUTION ON THE SPATIAL DIFFUSION OF *TRICHOGRAMMA CACŒCIAE*

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Tichogramma sp. are among the most studied biological control agents around the world and are used against a broad range of pest species. Nonetheless their use can still be improved and their behavior further understood. Specifically, the movement or dispersal of biological control agent is considered as one of the essential components for the success or failure in the field. The minute size of trichograms makes this particularly challenging to study. Most existing work addresses either very fine (behavioral) or fairly large (populational) spatial and temporal scales. We propose a novel experimental approach in laboratory conditions to understand the effects of population density, host presence and the spatial distribution of hosts on the diffusion and performance of *Trichogramma cacœciae*. We built an original experimental setting in which a 6-meter long linear arena is folded as a double spiral. By taking high-resolution pictures every minute and using dedicated image analysis methods, we could track individuals over several meters and eight hours, thus bridging the behavioral and populational scales. We showed that host presence had a positive impact on the diffusion capacity of trichograms regardless their spatial distribution. This result was not expected considering the significant time needed to parasitize a host. Furthermore, a higher initial density of individuals lead to a greater diffusion rate than a lower initial density, at least during the first four hours of the experiment. The presence of hosts in an environment could motivate individuals to keep searching during the whole experimental time. Without the hosts, we identified a diffusion threshold that could be explained by the lack of chemical or visual stimuli in an empty environment.

Keywords: Trichogramma, spatial diffusion, density, host distribution, image analysis

CO311

IMAGING BEE BRAINS TO HELP UNDERSTAND LEANING AND MEMORY

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The experiment was conducted as a “proof of principle” study to identify potentials for MicroCT scanning of live bee brains to be used as a non-invasive method for studying learning and memory. A bench-top MicroCT scanner and a synchrotron Beamline were used to scan live bees. To enhance tissue differentiation, bolus injections of radiographic contrast were delivered directly into the haemolymph. Brain volume and plasticity data were measured using BeeView software. Gross brain morphology was visualised in 2D and 3D projections. Scanning of live bees enabled minimally-invasive imaging of physiological processes (for the first time) such as passage of contrast from gut to haemolymph as well as preliminary brain perfusion and plasticity studies. Brain morphology results were correlated with learning responses in live bees. Understanding the relationships between experience and brain structure is key to understanding the relationships between learning and memory. Simple environmental manipulations can both accelerate and delay brain growth in bees, and since brain volume and density are sensitive to behaviour throughout life, the honeybee has great potential as a model for exploring the interactions between learning, memory and brain structure. The use of CT imaging for the non-invasive study of insects (termed Diagnostic Radioentomology-DR) is increasing (Greco *et al.* 2005; Greco *et al.* 2008; Eyer *et al.* 2015; Greco and Stait-Gardener 2017). Results from this experiment demonstrate limitations of live bee scanning however they also show the great potential for in-vivo, non-invasive DR imaging of the live honeybee for future research and teaching of brain morphology, physiology and function.

Keywords: Radioentomology, MicroCT, Bee Brain, Learning, Memory

CO312

NEW MORPHOLOGICAL TECHNIQUES AND BIG DATA IN ENTOMOLOGY: A CASE STUDY OF *MEGAPHRAGMA AMALPHITANUM* (HYMENOPTERA: TRICHOGRAMMATIDAE) PROVIDING THE FIRST SUBCELLULAR RECONSTRUCTION OF A COMPLETE INSECT EYE

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Many recently developed high-resolution morphological methods promise further breakthroughs in the study of insect morphology. At the same time, the high resolution of these methods often imposes limitations on the size of the studied organism. Therefore, miniature insects are of particular interest for such studies, because they possess all the physiological, cognitive and ethological capabilities found in larger insects and, at the same time, their smaller bodies allows complete reconstruction of all sensory organs using methods that can be applied to other insects only for small parts of one particular sense organ. This is why parasitoid wasps *Megaphragma amalphitanum,* which have a body size as small as 240μm, can be used as the key model organism for successful reconstruction of biological networks. We examined the head of *Megaphragma amalphitanum* under a dual beam microscope (FIB-SEM) with a isotropic resolution of 8 nm per voxel. Based on a large array of data, we prepared the first complete 3D reconstruction of the compound eye, including cells and subcellular structures. This reconstruction considerably contributes to our knowledge of the 3D organization and spatial orientation of cells in miniature compound eyes, to the conception of insect vision, and to our understanding of the processing of visual information in animals. Comparative morphological analysis shows a high degree of similarity of the eye of the studied wasp with that of the honeybee in some general points, such as the cell arrangement in the retinula and cell structure. In spite of this, the unique morphological adaptations that were described in the Megaphragma eye in our earlier study have also been confirmed. Analyses of cell volumes and of subcellular components have also been performed, yielding results that can be explained in the light of possible morphofunctional adaptations.
This study was supported by the Russian Foundation for Basic Research (project nos. 16-04-01627 and 17-04-00669).

Keywords: Miniaturisation, Megaphragma, insect, compound eyes, FIB-SEM

CO313

SYNCHROTRON BASED X-RAY TOMOGRAPHIC MICROSCOPY FOR ENTOMOLOGY: FROM EXTINCT INSECTS IN AMBER TO BUZZING FLIES

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Synchrotron-based X-ray tomographic microscopy is a powerful technique providing volumetric quantitative information on the internal structure of a wide range of bulky materials in a non-destructive manner at the micrometer scale. Synchrotron radiation as available at third generation sources has peculiar properties enabling unique experiments. The high brilliance coupled to latest developments in detector technology [1] pushes the temporal resolution of this technique to the sub-second level, paving the way for a wide range of time resolved applications and in-vivo experiments that were not possible before. The high beam coherence extends instead the palette of imaging techniques from the traditional methods based on absorption contrast to edge-enhancement and phase-sensitive measurements [2] boosting density sensitivity. The TOMCAT beamline [3] at the Swiss Light Source has established itself as a cutting edge hard X-ray tomographic microscopy endstation for experiments on a large variety of samples, such a new materials, biomedical tissues and rare fossils. Here we outline the features of the instrument and present a palette of recent applications in entomology covering different length and temporal scales. The examples include the analysis of morphological characters for updating phylogeny information [4], the study of extinct insects preserved in amber and in-vivo experiments unravelling complex insect behaviours, such as involved flight manoeuvres [5].

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Keywords: X-ray tomographic microscopy, synchrotron radiation, time-resolved experiment, nanotomography

CO314

NEURAL CODING OF OLFACTORY SPATIAL INFORMATION IN THE AMERICAN COCKROACH

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Animals rely on olfaction to navigate through complex olfactory landscapes, but the mechanisms that allow an animal to encode the spatial structure of an odorous environment remain unclear. To acquire information about the spatial distribution of an odorant, animals may rely on bilateral olfactory organs and compare side differences of odour intensity and timing or may perform spatial and temporal signal integration of subsequent samplings. In insects, cognate olfactory sensory neurons (OSNs) originating from different locations on the antenna, but bearing the same olfactory receptor, converge onto the same glomerulus within the antennal lobe, which is thought to result in a loss of spatial information. However, in the american cockroach *Periplaneta americana*, OSNs from different locations along the flagellum innervate the glomeruli in an antennotopic fashion, thus preserving the information on the spatial localization of a stimulus. Moreover, morphological and functional analysis on the macroglomerulus (MG), a larger glomerulus specialized in female sexual pheromone detection, revealed the existence of twelve pheromone-responsive projection neurons (PNs), each with a stereotypic subglomerular arborization and with a spatially tuned receptive field along the antenna. In addition, each PN type innervates a different compartment of the mushroom body, allowing to maintain spatial olfactory information along the olfactory circuit. In summary, the combination of (a) the antennotopic organization of OSNs terminals and of (b) the stereotyped compartmentalization of PNs' arborization both in the MG and in the MB, provides the first mechanism for encoding information on the spatial distribution of an odorant.

Keywords: Cockroach, olfaction, imaging, antennal lobe, olfactory receptor neurons, projection neurons

CO315

ANTENNAL GROOMING IN *NEZARA VIRIDULA* (HEMIPTERA, PENTATOMIDAE): FUNCTIONAL MORPHOLOGY OF THE TIBIAL COMB

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Grooming is a well-recognized, multipurpose, behavior in arthropods and vertebrates. In particular, insects self-groom their antennae to keep them clean and responsive to the environment. Insect cleaning systems include hairs or projections used to mechanically remove particles by scraping, transport and concentrate them. Antennal grooming has been investigated in different orders. Typically the cleaning organs are located on forelegs and mouthparts. In Hemiptera, tibial organs were described in many species only for taxonomic purposes and their role in grooming is still poorly investigated. The present study aims to describe the behaviour (observations under stereomicroscope using high speed camera) of *Nezara viridula* during antennal cleaning and the functional morphology (cryo-SEM and TEM) of the involved organs, in particular the tibial combs. Moreover, the presence and distribution of the elastic protein resilin in these structures is revealed by confocal laser scanning microscopy. We observed an involvement of the tarsal hairy pads (*N. viridula* attachment devices) in cleaning the tibial comb. To estimate different role of the cleaning devices, we evaluated the efficiency in removing particles using insects in different conditions (intact, with ablated tibial comb and with shaved hairy pads) with their antennae covered with pink dust. A better understanding of insect grooming may help to identify contaminants that insects cannot easily clean. This in turn can provide relevant information potentially useful for insect pest control, particularly important in the case of *N. viridula* which represents a serious pest of different crops in most regions of the world.

Keywords: Surface cleaning, functional morphology, biomechanics, stink bugs

CO316

ANATOMICAL NETWORKS AND GLOBAL BRAIN ACTIVITY

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Invertebrate models are widely used in order to understand how adaptation to ecological niches shaping brain structure and circuitry throughout evolution. In particular, for small insect brains, our knowledge of structural brain networks down to cellular and molecular level of microcircuits are far advanced. How can we use these anatomical maps to plan experimental approaches that allow analyzing the dynamic and function of local and global brain circuitry that eventually elicits behavior? On the mesoscale level, neural network analysis, using singe cell neuron morphologies, 3D digitized brain models and image registration allows hypothesis of how multi-channeled sensory information integrates in central brain circuits. Thus, central processing units (CPU) are proposed, in which sensory, multimodal and feedback circuitry are orchestrated. In the olfactory system, CPUs are formed by neuropil structures, the glomeruli. Newly developed, automated EM techniques, such as Focused-Ion-Beam Scanning Electron Microscopy (FIB-SEM), allows for full-scale connectomes of specified brain regions and complete reconstruction of local synaptic circuits including subcellular features. The functional connectivity in these precise anatomical maps, depicting global and local brain circuitry can be tested by in-vivo imaging experiments using fluorescent markers and fast 3D tomographic imaging methods, such as light sheet microscopy or high-resolution 3D lattice light sheet. A major obstacle in insects are non-transparent cuticle that must be overcome with clearing techniques in order to visualize dynamic activity in the brain.

Keywords: Olfactory glomeruli, Volumen-based Electronmicroscopy, Light Sheet Tomography, Brain Atlas, Microcircuits

Insect Morphology in the Age of Phylogenomics

CO317

THE EVOLUTION OF MANDIBULAR ARTICULATIONS IN EARLY SPLIT HEXAPODA

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The morphology of insect mandibles and their articulation with the head capsule is a frequently used character system in hexapod systematics. It is currently assumed that the primary wingless insect lineages (Protura, Collembola, Diplura, Archaeognatha and Zygentoma) exhibit two mandible types. Monocondylic mandibles are principally composed of one posterior articulation with the head while dicondylic mandibles show an anterior and posterior articulation. Although several studies already reported additional mandible “interactions” with the head in Collembola and Archaeognatha or clarified the structural composition of already known articulations, the phylogenetic interpretation of these structures and their implication for the biomechanical evolution of the food uptake system in insects remained unclear. Here, additional mandibular articulations in Collembola, Diplura and Archaeognatha are reported which illuminate the evolution of the food uptake system in insects. In addition to the posterior mandibular articulation, Collembola and Diplura each show an additional articulation of the maxillar stipes with the mandible. Archaeognatha in fact possess two anterior mandible articulations, one with the head capsule and another one with the first maxillar palpus segment in additional to the posterior ball-and-socket joint. Based on morphological comparisions and homologisation of the involved structures, it is proposed that mandible-maxilla joint is a homologous structure in Collembola and Diplura, while the anterior head-mandible joint in Archaeognatha is homologous to the anterior joint of Zygentoma. Archaeognatha are therefore dicondylic insects. Although these discoveries are difficult to interpret from a phylogenetic point of view, the character system mandible articulation is obviously more variable than previously assumed. The biomechanical implications of the different mandible articulations reported here are discussed.

Keywords: Collembola, Diplura, Archaeognatha, Zygentoma, monocondylic, dicondylic, biomechanics

CO318

THE SPERM ULTRASTRUCTURE OF COCCINELLIDAE (COLEOPTERA) REVEALS A GREAT DIVERSITY IN THE GROUP

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Coccinellidae is a large group of ladybird beetles that exhibits remarkable morphological and biological differences. Despite their ecological importance, the systematics and phylogenetic relationships between the subfamilies of the group is not yet satisfactory. According to Ślipiński (2007) and Seago *et al.* (2011) only two of the six subfamilies described by Sasaji (1968) would be recognized. A molecular phylogeny of Coccinellini by Escalona *et al.* (2017) supports the monophyly of the tribe and its sister group relationship to Chilocorini, but underlines that the classification of Coccinellini has been misled by convergence in morphological traits. The sperm ultrastructure of 12 species included in Coccinellinae, Chilocorinae, Scymninae and Epilachninae (Sasaji, 1968) was studied., The sperm of all members of Coccinellidae share the uncommon pattern of the posterior sperm region with the nucleus running parallel to the axoneme and other flagellar components. Three sperm types were recognized in this study. The first type, characterized by a long acrosome, a long basal body and thin mitochondrial derivatives, was shared by several species of Coccinellinae, irrespective to which tribe they belong; the second sperm type, characterized by a short acrosome, a short basal body and mitochondrial derivatives with median diameter, was found in the Coccinellinae *Coccinula quatuordecimpustulata* and *Tytthaspis sedecimpunctata*; the third sperm type, characterized by a long acrosome, a short basal body and thick mitochondrial derivatives, was found in the Chilocorinae *Exochomus quadripustulatus*. These results indicate that the generic concepts within Coccinellini do not reflect a natural classification. *C. quatuordecimpustulata* has a sperm organization different from other Coccinellinae, but it shows a similar structure of the basal body. On the contrary, *Cryptolaemus montrouzieri*, *Scymnus apetzi* and *S. marginalis* of the subfamily Scymninae, share the sperm type of Coccinellinae. Finally, the Chilocorinae *E. quadripustulatus*, exhibits a basal body with structure similar to that of insects in general.

Keywords: Insect sperm ultrastructure, sperm structure of beetles, insect systematics

CO318a

SIZE AND SENSIBILITY: A STUDY ON ANTENNAL SENSILLA OF MEGAPHRAGMA, A MINIATURE PARASITOID WASP

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Antennal sensory systems of insects are very diverse and complex, reaching a very high level of complexity in some groups. However, the extremely

small sizes of some species impose strict limitations, which affect antennal morphology in various ways. The genus *Megaphragma* (Hymenoptera: Trichogrammatidae) contains some of the smallest flying insects with body size in some cases as small as 170µm. Such extreme miniaturization affects them on organ, cellular and even sub-cellular levels. Nevertheless, their antennae remain fully functional, allowing highly specific host search and recognition. Our goal was to study the effects of miniaturization on antennal sensory system of Megaphragma and estimate the limits to its reduction. We examined the external morphology and distribution of the antennal sensilla of *M. mymaripenne*, *M. amalphitanum*, and *M. caribea* using scanning electron microscopy. We performed quantitative morphological analysis and compared our findings with data on the larger parasitoid wasps. Eight types of sensilla were distinguished: s. chaetica, s. trichodea (types I and II), s. basiconica (types I and II), s. styloconica, s. placodea and Böhm's bristles. The maximum number of sensilla in all three species studied was 41 per antenna in M. mymaripenne females. The overall number of sensilla and their types is significantly smaller than in related groups. Our results provide an insight into the effects of miniaturization and the associated morphological simplification of antennal sensory systems.
This study was supported by the Russian Science Foundation (17-74-10246).

Keywords: Parasitoid, miniaturization, sensilla, host location

CO319

THE FLIGHT OF FEATHERWING BEETLES (COLEOPTERA, PTILIIDAE) AND A NEW MECHANISM OF AIR LOCOMOTION OF TINY INSECTS

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The study of insect flight is a large branch of modern entomology, strongly linked to biomechanics and aerodynamics. Most publications in this area treat the flight medium-sized insects, and the flight mechanics is similar in the majority of studied insects. The question about the flight of microinsects at extremely low values of the Reynolds number was raised repeatedly, but experimental data on this problem are limited and are refer to insects that are much larger than the smallest. The smallest free-living insects are beetles of the family Ptiliidae. They have wings of the characteristic feathered shape, but it remained unknown whether they are capable of active flight or are passively borne by the wind. We have performed high-frequency video recording of the free flight of ptiliids, used 3D tracking to reconstruct the trajectories of their flight, and showed that ptiliids are capable of highly maneuverable flight at a velocity of up to 1.5 m/s. We have also reconstructed the movements of their wings, calculated the angles of attack, wing speed, and the Reynolds number. The wing cycle of these beetles is unique and consists of two rowing movements and two return movements. During the rowing movements the wing moves rather rapidly at high angles of attack. During the return movements both wings, after the beetle claps them together, are pressed to each other and relatively slowly carried above or below the body. Thus, the wing kinematics of ptiliids displays certain components typical of the movements of some aquatic invertebrates: it combines components of flight and swimming.
This study was supported by Russian Science Foundation (14-14-00208).

Keywords: Ptiliidae, Flapping flight, Wing kinematics

CO320

PHYLOGENY OF RHINOPHORIDAE AND POLLENIIDAE (DIPTERA): TOWARD THE EVOLUTION OF OESTROIDEA

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Calyptrates are a megadiverse, actively radiating, group of dipterans, which are widely spread and abundant in nearly all terrestrial environments. Despite huge diversity and economic importance, their phylogeny is far from resolved. Recent attempts employing few taxa seem converging in retrieving monophyly for most of the families and subfamilies, but deep relationships among these, especially for those of the oestroid clade (blow flies and relatives), are labile when not changing. The goal of the present project is to shed some light on the deep phylogenetic relationships among Calyptratae by using an anchored hybridization approach with a careful taxon sampling. Furthermore, we aim at resolving the generic phylogeny of two key families of parasitoid flies: Rhinophoridae and Polleniidae. Rhinophorids are interesting because of their peculiar parasitoid habit: they are the only insects having exploited crustaceans (Crustacea, Isopoda, Oniscidea) as hosts. Moreover, adult rhinophorids are difficult to recognize from other oestroids due to the lack of autapomorphies. Differently, however, the preimaginal instars present sound autapomorphies. Notwithstanding the several phylogenetic investigations conducted so far, the phylogenetic position of the rhinophorids is ambiguous and there is an impelling need of improving both taxon sampling and sequence data in order to gain a better resolution. In turn, the phylogenetic position of the polleniids as sister group of the Tachinidae is becoming consensus recently, but the phylogenetic relationships within the family are still unknown. In conclusion we aim to reconstruct a solid phylogeny of these groups in order to build up a stable and predictive classification of the Oestroidea.

Keywords: Phylogeny, Evolution, Diptera, Oestroidea, Parasitoids

CO321

INSECT PHYLOGENOMICS: CURRENT STATUS, CHALLENGES AND SOME IMPLICATIONS FOR MORPHOLOGY

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Enormous progress has been made recently in exploring the phylogeny and evolution of Insects. Molecular data sets have increased exponentially in the last years (transcriptomes and genomes) and "parallel evolution" of innovative morphological methods took place ("renaissance of insect morphology"). A highly efficient working pipeline and refined analytical methods were developed in the 1KITE project (www.1kite.org/), which is primarily aiming at a reconstruction of the phylogeny and evolution of the entire Hexapoda based on transcriptomes. This resulted in a study covering all orders of insects including rare and very rare key taxa (Misof *et al.* 2014), with phylogenetic analyses based on 1478 orthologous genes. It is safe to say that the suggested results are presently the state of the art in high level insect systematics, and the robust pattern is an excellent base for evolutionary interpretations. Major issues like the monophyly of Polyneoptera or interrelationships in the megadiverse Hemiptera and Holometabola are largely resolved. A controversial result, also in an ongoing follow-up project, is the non-monophyly of Paraneoptera. Some issues, which were seemingly intractable with morphological characters, remain problematic even with enormous taxon samplings and transcriptomic data in 1KITE follow-up projects: a) the relationships of the three entognathous orders Collembola, Protura and Diplura, b) the basal splitting events in Pterygota, *i.e.* the relationships between Odonata, Ephemeroptera and Neoptera, and c) the monophyly of Mecoptera and the placement of the families Boreidae and Nannochoristidae. An easy solution of these problems is not insight. However, an intensified cooperation between palaeontologists, morphologists working with innovative methods, and leading experts in bioinformatics and molecular systematists may lead to a robust solution in the near future.

Keywords: Insects, phylogeny, transcriptomics, 1KITE, morphology

CO322

REVISITING THE TIMESCALE OF INSECT EVOLUTION USING MONTE SAN GIORGIO FOSSILS

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Insects, the most diverse animal group on earth with an estimated 5.5 million species, have evolved an extraordinary range of traits, from flight and complete metamorphosis, to polyphenisms and advanced eusociality. Understanding the timeframe for their evolution is of considerable interest. Although the fossil record of insects is extensive, data are scarce for a number of key periods. One such period is that following the Permian-Triassic mass extinction event - recognized as the most catastrophic of all major extinction events that led to the disappearance of 80-96% of marine species and of 70% of terrestrial vertebrates. Insects are thought to be not excluded by this extinction event. We have recently discovered a remarkable collection of 240 million-year-old insect fossils from the Monte San Giorgio Lagerstätte (Switzerland-Italy). These fossils, in addition of having uniquely preserved soft tissues and internal organs (*e.g.*, components of the central nervous system and muscular bundles), backdate the origin of several insect lineages by up to 200 million years (*e.g.*, Archaeognatha: Machilidae and Hemiptera: Tingidae). Phylogenomic and molecular clock analyses were performed using seven key fossils from Monte San Giorgio as calibration points, plus 35 insect fossils previously identified as suitable for such analyses. The estimated origins of over than 40 major hexapod lineages were backdated by more than 50 Million years compared with previously reported ages. Besides, over 20 lineages are substantially shifted from their currently accepted origins following the Permian-Triassic mass extinction event, to well before this major event. Our results indicate key innovations such as flight and metamorphosis evolved significantly earlier than previously thought, and that the bulk of extant insect lineages survived through the Permian-Triassic mass extinction event.

Keywords: Insect phylogeny, insect evolution, fossil insects, Middle Triassic

CO323

Abstract moved.

CO324

A MULTILOCUS APPROACH TO EXPLORE THE PHYLOGENY OF A MEGA-DIVERSE TAXON: THE CASE OF HYCLEUS (COLEOPTERA, MELOIDAE)

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Molecular phylogenetic studies involving mega-diverse genera represent a major challenge in systematics. In this context, Hycleus, the most speciose genus of the beetle family Meloidae, with at least 430 species, represents an interesting model to explore the phylogeny of a mega-diverse taxon. The systematic of this genus has never been investigated, but its members were divided into three "sections" according to the mesosternal morphology, whereas some lineages have been detected according to additional morphological characters. Previous molecular studies pointed out the close relation among Hycleus and both genera Ceroctis (59 species) and Paractenodia (5 species), forming a complex of ca 500 species. In the attempt to study the phylogenetic relationships among the species of this mega-diverse taxon, we tested about one hundred species (92 Hycleus, 16 Ceroctis and 3 Paractenodia) with both mitochondrial (COI) and nuclear (CAD, 28S, ARGK, Wingless) markers. Our Maximum Likelihood and Bayesian-based results showed that the three Hycleus "sections" are polyphyletic and lack of a taxonomic value, whereas some of the groups morphologically described were confirmed and well supported. Furthermore, the monophyly of the complex Hycleus-Ceroctis-Paractenodia was verified, suggesting that a new taxonomic arrangement of these taxa is required. However, in most cases, the basal nodes of the trees remained unsolved, therefore, to better define the deepest phylogenetic relationships, the application of novel markers is required.

Keywords: Mega-diverse genus, Coleoptera, Meloidae, Hycleus, Molecular Phylogeny

CO325

EVOLUTION OF HEAD STRUCTURES IN COLEOPTERA

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The feeding apparatus of insects is crucial in the context of the biology and ecology of the species. Despite of the enormous diversity of Coleoptera (ca. 380.000 spp.), the structure of the mouthparts of adults is fairly conservative – in contrast to other groups of Holometabola or to Hemiptera, they are almost always of the biting type. Within the framework of this groundplan setting, various structural changes occur, allowing to adapt to an enormous variety of different feeding types and substrates, especially in the megadiverse Polyphaga. The main aim of our project was to investigate the cephalic morphology of beetles with different feeding types and body size. A special focus was on miniaturized saprophagous forms, such as Ptiliidae (Staphyliniformia) and Sphaeriusidae (Myxophaga). However, representatives of the suborders Adephaga and Archostemata were also taken into consideration, with predacious habits or a reduced role of feeding, respectively. The evolution of the feeding apparatus is discussed in the context of the basal splitting events in Coleoptera, *i.e.* the relationships of the suborders Polyphaga, Myxophaga, Adephaga and Archostemata. Our evaluation of extensive morphological data suggests that saprophagy is a groundplan feature of beetles, with the presumably ancestral feeding apparatus similar to the one observed in Sphaeriusidae (Myxophaga), Clambidae (Polyphaga) and also members of Staphyliniformia and some other polyphagan groups. Archostemata display modifications likely linked with a very limited food uptake, and Adephaga have specialized on carnivorous habits with preoral digestion. A far-reaching reorganization of the head does not take place in extremely miniaturized species of Coleoptera, such as for instance Ptiliidae. The complexity of the feeding apparatus can even be increased, and the effects on appendages, cephalic digestive tract and muscles are very limited, with simplifications largely restricted to the endoskeleton.

Keywords: Coleoptera, head structures, phylogeny, miniaturization

Evo-Devo Perspectives on Insect Morphology

CO326

MAJOR TRANSITIONS IN THE EVOLUTION OF THE SEGMENTATION PROCESS IN INSECTS

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Our understanding of arthropod body plan development stems from work on *Drosophila melanogaster*. In Drosophila, there is a relatively detailed model for the network of gene interactions that proceeds in a hierarchical fashion to define the entire segmented body. However, most arthropods generate segments through a sequential process wherein a dynamic process in the posterior growth zone is used to delineate new segments. Thus, we have focused on the growth zone of the milkweed bug *Oncopeltus fasciatus* as a model for a relatively conserved and typical process of segment generation. We present a morphological description of the growing germband throughout segmentation, complemented by cell division profiles and expression patterns of key genes. Our results offer a comparative view and allow a reconstruction of the major transitions in the evolution of the different segmentation modes in insects. We have shown that simultaneous segmentation most likely appeared before the origin of Holometabola, and has been lost several times. We now add details about the evolution of different modes of sequential segmentation. We present the cascade involved in differentiating new segments from a posterior growth zone, and show that a hierarchy reminiscent of the classical Drosophila hierarchy is found in sequential segmentation as well. The cascade begins with primary pair-rule genes followed by secondary pair-rule genes, which regulate segment polarity genes. The cascade is highly redundant and RNAi phenotypes of most genes are surprisingly minor. This hierarchy was most likely ancestrally of a single-segment periodicity. However, there is evidence for a two-segment periodicity in the differentiation of the segments after their formation in Oncopeltus, perhaps giving a hint to the origin of the pair-rule pattern found in Drosophila.

Keywords: Development, evolution, segmentation

CO327

ADAPTIVE AND GENETIC BASIS OF AN EXAGGERATED SECONDARY SEXUAL TRAIT

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Phenotypic variance is ubiquitous in nature and constitutes one of the major pillars of evolution by natural selection. Some extreme examples of phenotypic variance are manifested in the massive differences in growth and scaling relationships of certain body parts among individuals of the same population. *Microvelia longipes* is a hemimetabolous insect that displays a striking case of variance where males of the same population display extreme differences in the growth of their legs and body. Behavioral experiments determined that male rear legs are used as weapons to chase opponents of the same sex away from sites where females come to lay their eggs. Comparison of mating systems revealed that the increased intensity of fights in *M. longipes* compared to other Microvelia species is associated with the evolution of the rear leg exaggeration. Nutritional manipulation and artificial selection experiments demonstrated that this extreme growth is influenced by both heritable and environmental factors. To identify the developmental genetic basis of leg exaggeration in males, we generated a comparative transcriptome between legs, sexes and artificially selected lines in a developmental stage where the leg elongation is the most prominent. Knockdown using RANi of genes whose expression is strongly correlated with the leg exaggeration in males, revealed a decrease in rear leg length specifically in males *M. longipes*. This new model system, therefore, offers a good opportunity to understand the ecological and genetic mechanisms underlying the evolution and maintenance of exaggerated traits and high phenotypic variance in sexually dimorphic species.

Keywords: Sexual selection, Scaling relationships, Male-Male competition, Phenotypic plasticity

CO328

COMPARTMENT BOUNDARIES AND MORPHOGENS RESPONSIBLE FOR PATTERNING INSECT WINGS

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Decades of research on the highly modified wings of *Drosophila melanogaster* has suggested that insect wings are divided into two Anterior-Posterior (A-P) compartments separated by an axis of symmetry. This axis is created by a developmental organizer that establishes symmetrical patterns of gene expression that in turn pattern the A-P axis of the wing. This model has been projected onto the wings of other insect species. Yet Drosophila possesses highly modified wings compared to most insects: forewings are reduced in size and venation, while diminutive hindwings lack venation and have been converted to halters. Lepidoptera possess more typical insect wings and wing colour patterns that provide landmarks for studies of wing structure and development. Using eyespot colour pattern variation in Vanessa butterflies, an independent contract analysis identified an additional A-P axis of symmetry running between wing sectors 3 and 4. This axis coincides with an apparent compartment boundary evident from clonal boundaries in lepidopteran mosaic gyandromorphs. This suggests the existence of a previously undetected Far-Posterior (F-P) compartment boundary subdividing the posterior compartment originally identified in Drosophila. This boundary and an associated developmental organizer create an axis of wing colour pattern symmetry and a gene expression-based combinatorial code, permitting each insect wing compartment to acquire a unique identity and allowing for the individuation of butterfly eyespots. The extent to which this new model of A-P wing patterning from Lepidoptera applies to other insects is unknown, but boundaries of experimentally produced mitotic clones in Drosophila coincide with the F-P boundary identified in Lepidoptera. The evolutionary conservation of certain characteristic features of venation across all winged insects suggests that the F-P boundary may be broadly applicable to insect wings. This emphasizes the value of using multiple model systems for understanding the development of structures.

Keywords: Evolution and Development, Insect wings, Lepidoptera, Drosophila, gynandromorph, Vanessa

CO329

ANTENNAL SEGMENTATION AND THE PERIODIZATION OF INSECT DEVELOPMENT

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To describe and compare the events and the associated morphological changes occurring along the development of different insect species, we need to adopt a suitable periodization of ontogeny. However, we should be always aware of the fact that any periodization is necessarily based on prioritizing one or a few traits or processes and disregarding others upon which we would establish a different partitioning of developmental time, while acknowledging smooth continuity across a divide suggested by the privileged criteria. This is particularly critical in respect to the two main pillars of current periodization schemes, *i.e.* the transition from embryonic to post-embryonic life and the generally final moult with which the adult condition is reached. Less dramatic but also serious is the 'cuticular perspective' adopted in partitioning the post-embryonic segment of an insect's life into instars separated by moults. One of the many processes whose interpretation is seriously affected by the traditional periodization of development is the segmentation of the antennal flagellum. The segmentation mechanics of the insect antenna is most clearly visible in non-holometabolous insects with high numbers of antennomeres and high number of post-embryonic moults with which an increase in the number of flagellomeres is associated. However, several circumstantial threads of evidence convergently point to the suggestion that the process is the largely same, except for its temporal schedule, also in the less accessible developmental phases, *i.e.* the embryo and, in the case of holometabolous insects, the pupa.

Keywords: Heterochrony, periodization, antenna

CO330

THE DNA DAMAGE RESPONSE PATHWAY REINFORCES SOCIAL HARMONY IN ANT SOCIETIES

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A key feature of eusociality in ant colonies is their reproductive division of labor, where queens reproduce and workers perform most other tasks. However, in the majority of ant genera, workers have retained their ovaries creating a potential conflict between queens and workers over reproduction. In advanced ant societies, this conflict is reduced through an adaptive developmental mechanism called 'Reproductive Constraint,' which causes the mislocalization of mRNA's and proteins that regulate germline and axis specification in worker ovaries. This mechanism results in 'failed' eggs reducing production of viable embryos. At the same time, Reproductive Constraint facilitates production of trophic embryos for nutrition. Despite the importance of Reproductive Constraint in reducing conflict over reproduction and transforming ovaries into a food producing organ to feed the colony, little is known about its underlying molecular mechanism. Therefore, we examine the role of the DNA-damage response pathway, a highly conserved pathway that regulates fertility, in regulating Reproductive Constraint in worker ovaries. We uncovered differential regulation of the DNA-damage response pathway between queens and workers, suggesting that this pathway was coopted to regulate Reproductive Constraint in ant advanced ant societies.

Keywords: Ants, Eusociality, Oogenesis, Reproductive Constraint

Systematics, Biogeography and Ecology of Leaf Beetles
Third European Symposium on the Chrysomelidae

CO331

A REVIEW ON FLEA BEETLES (CHRYSOMELIDAE: ALTICINI) OF TURKEY WITH RESPECT TO SPECIES NUMBER AND HOST PLANT ASSOCIATIONS

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Turkey has a quite rich leaf beetle (Chrysomelidae) fauna when compared with its neighbors although many regions are not well studied yet. There are recently more than 900 reported taxa, of which about 350 belong to the tribe Alticini, with about 10% endemism ratio. This is surely a result of hotspots included in the area. Alticini, or flea beetles, is one of the largest taxon of the family Chrysomelidae and is distributed throughout the world. Adults and larvae feed on various parts of the herbaceous plants, bushes, and trees causing characteristic "shot holing" damage resulting in weak appearance, or reduced crop value. Some of the species are consistent pests, whereas several are beneficial due to their importance as biological control agents of weeds. In the present study, knowledge about flea beetles and their host plant associations in Turkey has been reviewed. Some generalizations were made based on personal field observations and other available literature. Despite several faunistic studies, knowledge on flea beetles and their host records is still insufficient in Turkey. Analyses of the recent flea beetle fauna in Turkey show that some species are closely associated with certain host plants while others are capable of occupying many others. In this respect Lamiaceae, Brassicaceae, Scrophulariaceae, Boraginaceae and Asteraceae are the mostly preferred host plant families in Turkey. Families such as Euphorbiaceae, Resedaceae, Solanaceae, Plantaginaceae, Cyperaceae, Convolvulaceae, and the others generally have less feeding records and numerous other plant families have no records at all. It is important to know the species numbers for evaluating the biodiversity of a particular region. From this point of view, the estimated number of Turkish flea beetle fauna is not accurate because there is still lack of data from many regions. More surveys and further efforts are needed to reach a reasonable level of completeness.

Keywords: Chrysomelidae, Alticini, fauna, host plant, Turkey

CO332

SYSTEMATIC AFFINITIES BETWEEN MEDITERRANEAN AND SOUTHERN AFRICA TAXA IN THE TRIBE ALTICINI: SOLVING A BIOGEOGRAPHIC PUZZLE USING MOLECULAR PHYLOGENETICS ON *LONGITARSUS* ASSOCIATED WITH BORAGINACEAE (COLEOPTERA CHRYSOMELIDAE)

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Longitarsus Latreille, 1829 is a very large flea beetle *genus* (Coleoptera, Chrysomelidae, Galerucinae, Alticini), widespread throughout all zoogeographical regions and including about 600 known species. *L. anchusae* species-group, occurring with four species mainly in the Mediterranean area, and the *L. capensis* species-group, occurring with 15 species in southern Africa, are both associated with the Boraginaceae plant family and with Mediterranean-type ecosystems. Based on the morphology, they result closely related for sharing several robust synapomorphies, including on genital structures such as aedeagus and spermatheca. This systematic affinity between Mediterranean and southern African taxa suggests a biogeographic and evolutionary puzzle that can be explained by two mutually exclusive hypotheses: (i) recent long-distance dispersal events from Mediterranean to southern Africa; (ii) stepping-stone dispersal events from the Mediterranean area through ""arid corridors"" in eastern Africa during Pleistocene glaciations. However, an alternative hypothesis could be to interpret the morphological similarities shared between Mediterranean and southern African taxa as a striking case of convergent evolution affecting also genitalia structures, rather than as synapomorphies due to a common recent ancestry. Here we try to disentangle theses hypotheses, shedding light on this biogeographic and evolutionary puzzle through a molecular phylogenetic approach.

Keywords: Chrysomelidae, Alticini, Longitarsus, Mediterranean area, South Africa, Boraginaceae, biogeography, molecular phylogeny

CO333

CHRYSOMELIDAE CHECKLISTS AND CATALOGUES – SYNTHESIS OF RESULTS AND BACKBONE FOR FURTHER RESEARCH

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We do not know yet how many species live on Earth. Estimation spans from 5 million minimum to 100 million. A first step is to know how many species are described yet. Therefore several institutions provided projects for checklists to list all valid species names of a specified taxonomic group in a specified area. Therefore you need the specialists of that group who can identify specimens, synonyms, erroneous and fake records, and who know the literature very well. The Austrian Academy of Sciences started a new series of Checklists of Austrian Animals in 2004. The first one - the prototype - was the checklist of the Chrysomelidae of Austria, where the criteria were elaborated for the whole series (https://verlag.oeaw.ac.at/artikel_2318.ahtml?page=product&info=2318). The Royal Belgian Institute of Natural Sciences started a project of global checklists of freshwater organisms which was also necessary for the management and protection of Freshwater ecosystems (http://fada.biodiversity.be). The checklist of the subfamily Donaciinae, published 2015, was compiled mainly from the Catalogue of Palaearctic Coleopterea (Vol. 6, 2010: Chrysomelidae), where several problems could be identified, which need further research. There are also a lot of questions remaining with the species outside of the Palaearctic. In 2017 the 3rd edition of the Fauna Aquatica Austriaca was published by order of the Austrian Federal Ministry of Agriculture, Environment and Water Management. It contains the animal species which were recorded from freshwater sites of Austria, with ecological estimations and limnological characteristics which are used for environmental studies (http://www.ecoprof.at/index.php/faunaaquaticaaustriaca.html). All these checklists did not only use the collected knowledge of researches invested so far in the taxonomic groups, but also showed gaps in our knowledge, which stimulate further studies.

Keywords: Systematics, Checklists, Chrysomelidae, Donaciinae

CO334

OVERCOME GLUCOSINOLATES AND RADIATE: HOW HOST SHIFTS TO BRASSICACEAE HAVE LED TO SPECIES DIVERSIFICATION

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Many species of the flea beetle genus *Psylliodes* LATREILLE (Chrysomelidae: Galerucinae) are associated with Brassicaceae plants containing the glucosinolate-myrosinase system as defense against herbivores. Upon tissue damage, glucosinolates are hydrolyzed by the enzyme myrosinase, and reactive isothiocyanates are formed that deter non-adapted herbivores. So far, it is unknown whether Brassicaceae are the ancestral host plants of Psylliodes beetles or whether species in this genus adapted to the glucosinolate-based defense and shifted to Brassicaceae. To answer this question we reconstruct the phylogeny of Psylliodes species using seven single copy nuclear genes. So far, our analysis is based on 36 of the 190 described Psylliodes species, including 21 species feeding on Brassicaceae, eight on Solanaceae, and two on Fagaceae. The Brassicaceae-feeding species form the most recent clade in the current phylogeny, showing there was a host plant shift to Brassicaceae followed by a species diversification. One exception is the species *P. kiesenwetteri* which feeds on Brassicaceae, but clades together with Fagaceae-feeding species, suggesting that Psylliodes species adapted at least twice to the glucosinolate-myrosinase system. To elucidate how Psylliodes flea beetles overcome this chemical defense, we analysed the metabolic fate of ingested glucosinolates in *P. chrysocephala*, a member of the most recent clade. Our results show, that *P. chrysocephala* is not able to fully prevent the hydrolysis of glucosinolates by the plant myrosinase, and detoxifies isothiocyanates by conjugation with glutathione. Interestingly, *P. chrysocephala* can sequester and detoxify about 25% of the total amount of ingested glucosinolates, and thus evolved specific strategies to prevent the formation of isothiocyanates. The next step is to trace the molecular evolution of these strategies across the phylogeny, to determine the key adaptation that enabled Psylliodes species to feed and diversify on Brassicaceae plants.

Keywords: Psylliodes, host-plant shift, glucosinolate detoxification, coevolution, plant-insect interactions, phylogenetic analysis, adaptive radiation

CO335

PHYLOGENETIC IMPULSE TO LARGE-SCALE BIODIVERSITY RESEARCH IN THE TROPICS

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In the scenario imposed by the biodiversity crisis, there is an urge for efficient approaches to accelerate both the characterization and understanding of often complex and very rich communities of organisms, particularly in the tropics. In this talk, I will advocate for the use of robust and efficient molecular phylogenetic approaches to help us delimit and identify species in the tropics, enhancing our inventories and knowledge of species interactions. A couple of examples drawn from my own tropical leaf beetle community studies will illustrate my points.

Keywords: Biodiversity, phylogeny, species delimitation, species identification, tropics.

CO336

THE PHYLOGENETIC INFERENCES AND EVOLUTION OF CHRYSOMELIDAE BASED ON MITOCHONDRIAL GENOMES (COLEOPTERA: CUCUJIFORMIA: CHRYSOMELOIDEA)

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The Chrysomelidae is the one of the most diverse lineage of Coleoptera, and is composed of 12 or 13 subfamilies according to the recent classifications depending on the status of the flea beetles (Alticinae). Both morphological and molecular data now widely support three main clades, chrysomelines, eumolpines and sagrines. However, the phylogenetic relationship between these lineages remains unclear, while some small divergent groups, such as Spilopyrinae, Synetinae and Timarchini, cannot be associated confidently to either of these lineages. We revisited the higher-level phylogeny of the Chrysomelidae using full mitochondrial genomes for 108 taxa covering all subfamilies. The analysis mostly confirms the composition of the three major clades, but with some critical rearrangements within these, especially with regard to the position of the two monocot feeding subfamilies, Donaciinae and Criocerinae, which are branching in more basal positions. We also used recently discovered fossils from Burmese amber to calibrate the mitogenome tree, which indicated an early origin of the Chrysomelidae that pre-dates or coincides with the early evolution of angiosperms, unlike some previous analyses inferring a more recent origin. Mitochondrial genomes thus are a powerful marker that solve the basal

relationships of Chrysomelidae, and the ease of generating these data with modern sequencing technology will consolidate the classification at various hierarchical levels.

Keywords: Chrysomelidae, mitochondrial genomes, high-level phylogenetic relationship, divergent time

CO337

PRELIMINARY ANALYSIS ON THE COLONIZATION OF THE POTATO BEETLE POPULATIONS IN TURKEY USING MICROSATELLITE MARKERS

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Potato beetle, *Leptinotarsa decemlineata*, is the most important pest of potatoes in potato growing regions in the worldwide. Turkey is an important country in potato cultivation with different cultivars. Despite the fact that potato beetle is an important agricultural pest in Turkey, the state of its colonization and its route are uncertain. In this study, we used five nuclear microsatellite markers to obtain information about the population structure and invasion route of potato beetle populations in Turkey. Adult beetle samples were collected from 25 sublocations covering potato growing regions in Turkey. Bayesian-based BAPS program was used to reveal genetic differentiation based on population. Analysis grouped six different population groups into three different clusters: the first cluster formed Central Anatolia and Mediterranean populations. The second cluster formed Aegean, Thrace and Black Sea populations. The third cluster was Marmara population. Nei's genetic distance was used to construct a dendrogram using the unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis. The dendrogram similarly grouped the six populations into three distinct clusters. Although the factorial correspondance analysis shows a similar genetic grouping, there is admixture between the Central Anatolia population/Marmara population and the other group (Aegean, Thrace and Black Sea populations). Our analysis show that Central Anatolian population may play a basic role in the distribution of populations to other regions.

Keywords: *Leptinotarsa decemlineata*, microsatellite, genetic diversity, Turkey

CO338

PHYTOPHAGA 100: RETRACING THE EVOLUTION OF PLANT CELL WALL DEGRADING ENZYMES IN BEETLES OF THE PHYTOPHAGA

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Our world is dominated by green plants whose cell walls are mainly composed of several polysaccharides including cellulose, hemicelluloses and pectins. These polysaccharides represent the most abundant polymers on the planet and a rich source of energy to any organism which has evolved the ability to degrade them. Microorganisms, especially plant pathogens, are very efficient in utilizing these polysaccharides because their genomes possess genes encoding a range of so-called plant cell wall degrading enzymes (PCWDEs). In contrast, the paucity of animals possessing the ability to degrade these polymers is striking. Recently, the ability of herbivorous beetles from the Chrysomeloidea and Curculionoidea to break down these polysaccharides has begun to be appreciated. The presence of PCWDEs in the beetle's digestive tract, mostly encoded by endogenous genes, makes this degradation possible. Our work combines transcriptome/genome mining to phylogenetic analyses of beetle-derived PCWDEs, associated with their functional characterization. Our primary data show that the evolution of PCWDEs in Phytophaga beetles is very complex: (i) most of PCWDE-encoding genes have been originally acquired in Phytophaga beetles from microbial donors through several horizontal gene transfer events; (ii) the biological function of these protein families - substrate specificity or loss of enzymatic capabilities - evolved through subfunctionalization/neofunctionalization events. Our goal is to draw a close to exhaustive picture of the origins and the evolutionary history of the gene families encoding PCWDEs in beetles of the Phytophaga. In this context, I will introduce a recently funded project aiming to sequence the gut transcriptome of a hundred beetle species covering almost all subfamilies of the Phytophaga clade.

Keywords: Chrysomeloidea, Curculionoidea, transcriptome, Plant cell wall degrading enzymes

CO339

CHANGES IN LEAF BEETLE DISTRIBUTION IN CENTRAL EUROPE OVER THE PAST 100 YEARS

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On the basis of ca. 170.000 records of *Chrysomelidae s.l.* in Central Europe kept in the database ChryFaun, we analysed possible changes of beetle distribution areas over the past 100 years. Of the 890 leaf beetle species covered in ChryFaun, we found evidence for area changes in 226 species between 1900 and 2017. 25 species have a smaller distribution area, 26 expanded their range northwards, 110 eastwards, 12 species southwards, 18 expanded to the West and to the North-East, 11 to the North-West, 19 to the South-East, and 5 to the South-West. Anthropogenic factors like land use as well as climate change are possible drivers of the observed changes. For certain areas in Central Europe, especially of the eastern part, more data are urgently needed.

Keywords: Coleoptera, Chrysomelidae, faunistics, area changes, database

Session 12.
Parasitology, Pathology and Immunity

Immune Defences and Virulence Strategies

CO341

P400 IS ANTIVIRAL AND REGULATES THE SIRNA PATHWAY IN *AEDES AEGYPTI* MOSQUITOES

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Aedes aegypti female mosquitoes are vectors of arboviruses such as Zika virus (ZIKV) and chikungunya virus (CHIKV). *Ae. aegypti* mosquitoes possess different immune pathways of which the major small interfering exogenous (si)RNA pathway that limits arbovirus replication. To identify new antiviral genes in Aedes, we performed a screen in mosquito cells on candidates previously shown to be involved in the siRNA pathway in the fly *Drosophila melanogaster*. Among candidate genes, we identified the gene p400 (also called domino) as antiviral in mosquito cells and also *in vivo* in *Ae. aegypti* females. Moreover, p400 is regulating *in vitro* and *in vivo* the expression of argonaute-2 (ago-2), a key player of the siRNA pathway, strongly suggesting that p400 is antiviral by mediating the exogenous siRNA pathway. Tissue-specific analysis of p400 and ago-2 gene expression and immunofluorescence assays show that p400 and ago-2 are both expressed in the same different female tissues. Interestingly, p400 regulates the siRNA pathway in a tissue-specific manner. These findings provide a novel insight into the regulation of the aedine antiviral exo-siRNA pathway.

Keywords: mosquito, arbovirus, antiviral immunity, siRNA pathway

CO342

ZOMBIE-FLY IMMUNE RESPONSE AGAINST A BEHAVIORALLY MANIPULATING HOST-SPECIFIC FUNGAL PATHOGEN

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Some Insect-pathogenic fungi (IPF) are generalists capable of infecting many different orders of insects, whereas other IPFs are highly host-specific naturally only infecting a single host species. In addition, IPFs range from obligate dependence on their host for growth and sporulation to facultative insect-pathogens that also occur in the soil and/or as endophytes inside plants. Pathogen specificity and degree of host dependence are important factors that shape host-pathogen coevolution and influence fungal virulence strategies and host immune responses. Most studies of insect immune responses towards IPFs have used facultative and generalist hypocrealean fungi (Ascomycota) with a wide host-range. Many of these fungi rely on fungal toxins to successfully infect and kill insect hosts and consequently elicit a significant host immune response. In contrast, many early-diverging IPFs in the subphylum Entomophthoromycotina (formerly part of Zygomycota) are obligate and highly host-specific insect pathogens. The IPF, *Entomophthora muscae*, grows as wall-less protoplast cells, presumably to avoid detection by the host immune system before taking over the behavior of the obligate host, the house fly (*Musca domestica*). Here, dual-RNAseq data of host and pathogen from experimentally infected house flies coupled with qPCR gene-expression and insect-immune assays are used to characterize the house fly immune response to *E. muscae*. Fungal infection results in the upregulation of several immune-related genes in house flies, showing that *E. muscae* is not able to completely avoid detection by the host immune system. Furthermore, the transcriptional analyses identify a set of unknown taxon-specific house fly genes that potentially are involved in a species-specific immune response towards *E. muscae* infection. Finally, the house fly immune response towards *E. muscae* is distinct in comparison to the immune response towards the facultative and generalist IPF, *Beauveria bassiana*, which highlight the many insights of insect immunity that can be gained from studying host-specific IPFs.

Keywords: Insect immunity, host-pathogen evolution, dual-transcriptomics, Insect-pathogenic fungi, Entomophthorales

CO343

GENETIC VARIATION IN THE SUSCEPTIBILITY OF DROSOPHILA TO VIRAL INFECTION

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Insects are infected with a diverse array of viruses in nature. Within populations it is common to find extensive genetic variation in susceptibility to infection. We have found that two different insertions of a transposable element are associated with resistance to viral infection (DAV and the sigma virus). These insertions are both into the coding sequence of genes. These genes previously had no effect on viral replication, but chimeric transcripts containing sequence from the host genes and the transposable element are strongly antiviral. This is a new and somewhat puzzling form of antiviral defence.

Keywords: Drosophila, viruses

CO344

SYSTEMATIC FUNCTIONAL ANALYSIS OF DROSOPHILA ANTIMICROBIAL PEPTIDES USING CRISPR/CAS9 MUTANTS

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Antimicrobial peptides (AMPs) are universal effectors of innate immune systems. Their mechanisms of action have been studied extensively *in vitro* with the hope of developing novel therapeutic agents. *Drosophila melanogaster* is a powerful model to study innate immunity. Systemic infection triggers the production of a diverse array of AMPs by the fat body under the control of the Toll and Imd NF- B signaling pathways. Despite their extensive use as a readout of immune signaling, the contributions of each AMP to Drosophila host defence is poorly understood. It is also unclear whether AMPs function as a cocktail, or have specific roles in host defence. In addition, a number of studies have revealed non-immune functions for Drosophila AMPs, including involvement in aging and neurodegeneration. We took advantage of recent gene editing technologies to delete each known AMP of Drosophila (Attacin, Cecropin, Diptericin Drosocin, Drosomycin, Metchnikowin and Defensin). Using single and multiple knock-outs, as well as a variety of infectious agents, we characterize the *in vivo* function of individual and groups of AMPs. This study explores the concepts of AMP specificity and AMP synergy in an *in vivo* setting, and highlights key interactions amongst AMPs and pathogens.

Keywords: Drosophila, Immunity, antimicrobial peptides

CO345

GLOBAL ANALYSIS OF THE VIROME IN *AEDES AEGYPTI* MOSQUITOES: ARTHROPOD BORNE-VIRUSES AND BEYOND

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Insects are the most abundant group of multicellular organisms and carry an incredible diversity of viruses. As such, insects can be seen as major reservoirs of viral biodiversity that can also be transmitted to other hosts. Therefore, the collection of viruses (*e.g.* virome) circulating in insects is of special interest. For example, we have seen many recent worldwide outbreaks by viruses transmitted by mosquitoes, such as Dengue and Zika. In addition, other components of the insect virome might not directly infect vertebrate hosts but could affect transmission of arboviruses by modulating vector competence. Finally, characterizing the incredible diversity of insect viruses could lead to the discovery of novel genes that can be tapped for biotechnological applications. Considering the importance of insect viromes, our group has used small RNA-based metagenomic approaches to identify and characterize viruses in different insects but specially *Aedes sp* mosquitoes. This strategy has important ad-vantages such as the fact that viral sequences are naturally enriched in the small RNA fraction of insects. In addition, the size distribution of small RNAs allows classification of viral sequences independent of homology searches against reference databases. Small RNA based metagenomics has greatly improved our ability to detect and identify novel viruses in insects thus allowing very detailed surveillance of insect viromes.

Keywords: Insect, viruses, virome, mosquitoes

CO346

A NOVEL, REPRODUCTIVE TISSUE SPECIFIC, INNATE ANTIVIRAL DEFENSE IN DROSOPHILA

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Arthropod-borne viruses are an increasing worldwide health concern. RNA interference (RNAi) is the main and best studied invertebrate antiviral response. In Drosophila, the siRNA pathway efficiently controls viral replication in somatic tissues. Other innate immunity or stress pathways also contribute to the antiviral defense, but their role and tissue specificity is less well characterized. The follicular somatic cells (FC) of Drosophila, an epithelial layer that encapsulates the germline and gives rise to the egg shell, is the last layer of somatic tissue blocking strict vertical transmission of pathogens. In FCs, independently of RNAi, the replication of a Flock House Virus (FHV)-derived replicon is partially repressed, and can only be observed in discrete patches. No piRNAs of viral origin are detect, excluding a role for the piRNA pathway as an additional viral control mechanism in reproductive tissues. Viral positive strand and the RNA dependent RNA polymerase (RdRP) are homogenously expressed, indicating a repression at the level of the RdRP activity. Replicon expressing cells showed decreased expression of several mitochondrial, fatty acid metabolism and ribosomal genes. *in vivo* RNAi assays of the candidate genes did not alter the pattern of replicon expression. This indicates that the observed changes in gene expression are a downstream result of differences in replicon activity between restrictive and permissive cells. An unbiased deficiency screen revealed further candidate genes. Their role on the control of the replicon will be discussed. Our results reveal an unexpected independence of known antiviral mechanisms and point to a yet unidentified mechanism controlling viral replication in FCs.

Keywords: Drosophila; Innate Antiviral Immunity; Follicular Cells

CO347

PERSISTENT INFECTIONS AND ANTIMICROBIAL PEPTIDE COMBINATIONS

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The simultaneous expression of costly immune effectors such as multiple antimicrobial peptides is a hallmark of innate immunity of insects, yet the adaptive advantage remains unresolved. Here, I address current hypotheses on the evolution of such defence cocktails in the beetle *Tenebrio molitor*. After inoculation with *S. aureus* a defensin and a coleoptericin are highly expressed *in vivo*. The defensin displays strong *in vitro* activity but no survival benefit *in vivo*. The coleoptericin provides a survival benefit *in vivo*, but no activity *in vitro*. To investigate this paradox we experimentally evolved *S. aureus* to increased resistance against the defensin and a combination of the defensin and coleoptericin. Genome re-sequencing showed that resistance was associated with a small number of specific costly mutations under both AMP treatments. All resistant lines display AMP cross-resistance. Costly resistance against AMPs readily evolves for an individual AMP as well as a naturally occurring combination *in vitro* and provides broad protection against AMPs. Such non-specific resistance could result in strong selection on host immune systems that rely on cocktails of AMPs. *in vivo*, the cocktail the expression of multiple AMPs increases host survival, contributes to the control of persisting infections and tolerance and reduces the risk of *in vivo* resistance evolution.

Keywords: Antimicrobial peptides, persistent infections, cocktail

CO348

GROWTH REGULATION OF THE ANTIVIRAL SYMBIONT WOLBACHIA

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The resistance of insects to pathogens is often modulated by their microbial symbionts. Wolbachia is arguably the most common endosymbiont in insects and can confer strong antiviral protection. This protection may partly explain why Wolbachia is so successful in invading new host species and is being applied in new interventions to fight against arboviruses, such as dengue and Zika viruses. Regulation of Wolbachia levels is a crucial aspect of their biology given that they are maternally transmitted. Higher titres increase transmission fidelity and strength of phenotypes, including antiviral protection. On the other hand, high levels of the endosymbiont carry a cost to the host and may be pathogenic, impacting host reproductive fitness and, therefore, harming Wolbachia transmission to the next generation. We are studying Wolbachia growth regulation in *Drosophila melanogaster* and its impact in antiviral protection. By analysing natural variation in Wolbachia we have been identifying symbiont genes involved in growth. Recently we have also developed a method to screen for overproliferate Wolbachia variants. We have isolated and genomically characterized two new such variants, and we are now investigating their induced antiviral resistance. These methods allow us to link genotypes to phenotypes in this unculturable endosymbiont.

Keywords: Endosymbionts, Wolbachia, Drosophila, viruses

CO349

VIRUS AND HOST-DERIVED SMALL RNAS IN VECTOR MOSQUITOES: IMPLICATIONS FOR HOST DEFENSE AND GENE REGULATION

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Antiviral immunity in insects is largely dictated by small interfering RNAs (siRNAs) which guide Argonaute proteins to complementary viral RNA sequences to induce their degradation. In Aedes mosquitoes, an additional, independent small RNA pathway processes viral RNA into PIWI-protein interacting RNAs (piRNAs). This is especially intriguing since in other model organisms piRNAs are almost exclusively derived from transposable elements, mobile genetic sequences that pose a threat to genome integrity due to random integration events. The *PIWI* gene family has greatly expanded in Aedes mosquitoes, raising the possibility that functional specialization allowed the pathway to process different RNA substrates. Indeed, piRNAs are produced from several other non-canonical substrates in Aedes, including protein-coding genes and Endogenous Viral Elements (EVEs), sequences derived non-retroviral RNA viruses that are integrated in mosquito genomes. Biogenesis and functions of viral and host-derived small RNAs will be discussed.

Keywords: *Aedes aegypti*, arbovirus, antiviral immunity, RNA silencing, RNA interference

Insect Gut Immunity and Microbiome

CO350

EXPLOITING THE MOSQUITO MICROBIOTA FOR DISEASE CONTROL

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Mosquitoes interact with a variety of microbes, and have a diverse intestinal microbiota, which plays essential roles in a variety of the insect's physiological functions. These microbes can also influence vector competence for human pathogens in multiple ways, including immune system activation or suppression, direct inhibition of the pathogen, and mosquito killing. Exploration of microbe-based anti-pathogen and entomopathogenic activities can provide essential knowledge for the development of novel disease-control approaches based on blocking pathogen infection in the mosquito and in the vertebrate host, and/or shortening the mosquito's life span. We will discuss and provide mechanistic insights on the negative and positive effects of natural environmental microbes on mosquito vector competence.

Keywords: Anopheles, Aedes, microbiota, malaria, dengue

CO351

HONEY BEE GUT MICROBIOTA - A VERSATILE MODEL TO STUDY MICROBIAL SYMBIOSIS

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Specialized bacterial communities colonize the gut of most animals. Despite their importance for host health, their complex composition presents a veritable challenge for elucidating fundamental aspects of gut microbiota function, ecology, and evolution. In my lab, we study the honey bee gut microbiota, a surprisingly simple, yet conserved gut community that is experimentally amenable and shares striking parallels to the mammalian system. Our overall goal is to combine experimental and genomic approaches to systematically understand functioning and evolution of this specialized, host-associated community. Metabolic activities of the microbiota are key for symbiotic interactions in the gut and directly impact health and disease of the host in manifold ways. In a recent study, we applied metabolomics to characterize the overall metabolic output of the microbiota in the honey bee gut. Besides identifying metabolic changes induced by the entire community, we also disentangled the contribution of individual microbiota members. Our results show distinct roles of bee gut symbionts in the conversion of major pollen wall constituents and the production of host-derived metabolites possibly involved in bee immunity and physiology. While we found evidence for cross-feeding interactions, a large fraction of the identified metabolic changes could be attributed to individual community members. Our study reveals diverse bacterial functions that are likely to contribute to bee health and provide fundamental insights into how metabolic activities are partitioned within gut communities. Future work needs to address how the identified metabolic changes impact the honey bee host.

Keywords: Gut microbiota, honey bee, metabolomics, evolution, symbiosis

CO352

THE EFFECT OF THE SYMBIONT ASAIA ON IMMUNE-RELATED GENES OF MALARIA VECTORS *ANOPHELES GAMBIAE* AND *ANOPHELES STEPHENSI*

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Mosquitoes hold a complex microbiota and that of several malaria vector species are particularly well characterized. Asaia, an acetic acid bacterium, is the dominant bacterium in the Asian malaria vector *Anopheles stephensi* while is just one of the components of the microbiota of *An. gambiae*, the main Afro-tropical malaria vector. This bacterium is a very promising candidate for a paratransgenic approach in malaria control. In light of field applications, we evaluated the growth trend of Asaia in *An. stephensi* and *An. gambiae* mosquitoes and the expression of major immune genes, namely CEC, DEF, CTL4, TEP1, known to be involved in Plasmodium surveillance. After emerging, mosquitoes were fed with three different diets: sugar 5%, sugar 5% plus 10^4 cells/ml Asaia and sugar 5% 10^8 cells/ml Asaia. One cage per each diet were fed only with sugar or sugar + Asaia, whereas to seven day old mosquitoes, blood meal was administrated as uninfected blood meal or as *P. berghei*-infected blood meal. Immunity gene expression analysis was conducted on ten mosquitoes per each cage at six different "post-emerging" check-points: 1, 3, 7, 8, 10 and 12 days. Our data showed dynamics of Asaia infection within the two malaria vectors. Asaia reaches homeostasis at day 3 in *An. stephensi* and at day 7 in *An. gambiae*, while its load seems to be regulated by different genes in the two species: in *An. stephensi* the regulation is almost completely dependent on the CTL4 activity, while *An. gambiae* a synergistic action of the CEC1, DEF1 and CTL4 seems involved. Additionally, a reduction of Plasmodium load was detected only in *An. stephensi* mosquitoes compared to the control group (newsworthy, in *An. gambiae*, P. berghei has a slower development compared to *An. stephensi*). All together these results suggest that Asaia is able to interfere with parasite development.

Keywords: Asaia, Plasmodium, mosquito immune system

CO353

SYSTEMIC MICROSPORIDIAN INFECTION+ENVIRONMENT=BACK TO ENTEROCYTES

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Our journey will start with bacterial intestinal infections in the model host *Drosophila melanogaster*. The next stage will bring us to consider intracellular parasitism. We shall then move on to honey bee collapse disorder and the connections between infections and environment. Next, we shall decipher how a nowadays famous compound in Europe interacts with flies and our journey after a detour through the nervous system will bring us back to the intestine. Our findings open a novel field of investigation of hitherto unknown defense mechanisms effective against occasional contamination of food.

Keywords: *Drosophila melanogaster*, intestinal host defenses, enterocyte purges, microsporidia, Serratia marcescens, pore-forming toxins

CO354

HERBIVORE INDUCED PLANT VOLATILES INFLUENCE ON GUT MICROBIOTA COMPOSITION AND INSECT SUSCEPTIBILITY TO ENTOMOPATHOGENS

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Multitrophic interactions involving insect pest, their natural enemies, environmental microorganisms and plant hosts are getting an increasing interest as relevant factors in pest management. In response to herbivory attacks, plants activate a wide range of defences that aim to reduce their damages. Attacked plants release herbivore-induced plant volatiles (HIPVs) which can act as priming signals to other plants, attract herbivore natural enemies and have a direct negative impact on herbivore survival. However, there are no reports on the possible influence of these plant secondary metabolites on the interaction of herbivore pests with microorganisms, including bacterial and viral entomopathogens. In the present work, we have tested the hypothesis that exposure to HIPVs renders insects more susceptible to natural pathogens. Exposing caterpillars of the noctuid *Spodoptera exigua* to indole and linalool, but not exposure to (Z)-3-hexenyl acetate increased the susceptibility to its nucleopolyhedrovirus (SeMNPV). We also found that exposure to indole, but not exposure to linalool or (Z)-3-hexenyl acetate, increased the pathogenicity of *Bacillus thuringiensis*. Additional experiments revealed significant changes on gut microbiota composition after forty-eight hours of larval exposure to indole. This result reveals that plant volatiles might influence herbivores ecology by modulating their interaction with pathogenic and non-pathogenic microorganisms.

Keywords: Plant volatiles, baculovirus, microbiota

CO355

PEPTIDOGLYCAN RECOGNITION PROTEIN-SD MODULATES HOST-MICROBE INTERACTIONS IN DROSOPHILA GUT

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Activation of the innate immune response in Metazoans is initiated through the recognition of microbes by the host pattern recognition receptors (PRRs). Peptidoglycan recognition proteins (PGRPs) are conserved innate immune molecules that function as PRRs and effectors. While the role of PGRPs as sensors of pathogenic microbes is well studied, their contribution to the sensing of commensal bacteria and establishing immune tolerance to microbiota is less clear. Using *Drosophila melanogaster* as a model, we study how PGRPs, specifically PGRP-SD, shape host-microbe interactions in the gut. Previously, we identified Drosophila PGRP-SD as a secreted pattern recognition receptor, which enhances IMD pathway activation in response to systemic infection (Iatsenko *et al.*, 2016). In the present study, we found that similar to its role in the fat body, PGRP-SD is also required in the gut for IMD pathway activation and defense against pathogens. Our data demonstrate that PGRP-SD, by inducing the expression

of negative regulators of IMD pathway in the gut, facilitates the establishment of tolerance to gut commensals and prevents the systemic spread of immune response. We discovered that PGRP-SD mutants exhibit reduced lifespan and gut dysplasia due to overproliferation of the dominant microbiota member *Lactobacillus plantarum*. We then deciphered the mechanism by which increased *L. plantarum* load leads to dysplasia and lifespan shortening. We identified *L. plantarum*-derived lactic acid as an inducer of NOX-dependent ROS generation in the gut, causing dysplasia and shortening the lifespan. These results identify PGRP-SD as a crucial factor regulating host-microbe interactions in Drosophila gut and uncover molecular mechanisms behind dysbiosis-induced lifespan shortening.

Keywords: Microbiota, Drosophila, dysbiosis, PGRP, lifespan, immune response, ROS

CO356

GUT MICROBIOTA COMPOSITION IS RELATED WITH THE PRESENCE OF VIRUSES IN FIELD CAPTURES FROM *SPODOPTERA EXIGUA* (HÜBNER)

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The gut microbiota plays an essential role in the metabolic, physiological and immune processes in all organisms. In addition, microbiota composition seems to influence on the interaction with pathogenic microorganisms. A previous study using laboratory-reared insects showed that changes in the gut bacterial load of *Spodoptera exigua* larvae affects the baculovirus insecticidal properties. The present work is a further study to evaluate the possible effect of the gut microbiota in the interaction of *S. exigua* larvae with its viral pathogens under field conditions. *S. exigua* larvae were collected from different greenhouses in the south of Spain during the 2015 and 2016 seasons. The microbiota composition and the presence of different entomopathogenic viruses (baculovirus and iflaviruses) in each larvae were determined and the possible correlation between the microbiota and viral infections was evaluated. Results from the microbiota composition analysis revealed that the bacterial load varies widely, and the bacterial composition is highly heterogeneous among individuals, regardless of the presence of viruses. However, some bacterial groups can be associated to the presence of viruses in the field. Two OTUs (Operational Taxonomic Units) from the genus *Acinetobacter* and *Pedobacater* have been found more abundant in the baculovirus infected samples, while *Ochrobactrum* has been related with the presence of iflavirus infection. Our results suggest an influence of the microbiota in the ecology of the viral infections and offer valuable information that could be useful for the management of insect pests using entomopathogenic viruses.

Keywords: Nucleopolyhedrovirus, iflavirus, microbiota, 16S, OTU

CO357

INTERPLAYS BETWEEN REPRODUCTIVE PHYSIOLOGY AND ANTIVIRAL IMMUNITY IN THE MOSQUITO *AEDES AEGYPTI*

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Mosquito-borne infections remain a worldwide burden with a continuous expansion into new geographic areas and epidemics such as outbreaks of chikungunya and, more recently, Zika viruses. The efficiency of mosquito-borne disease transmission under natural conditions is referred to as the vectorial capacity which depends on many factors including mosquito reproduction and vector competence. For this reason, control of mosquito vectors either by mosquito population reduction or replacement with refractory mosquitoes can play an important role in the fight against transmission of mosquito-borne pathogens. Female of some mosquito species can act as vectors because they need a blood meal from a vertebrate host to develop their eggs. Thus, whilst a female mosquito is first infected with a pathogen and initiates an immune response, she also undergoes tremendous physiological changes at the molecular, cellular, tissue and systemic levels for her reproduction. It is unknown how vector reproduction and competence affect each other while this is of importance to model transmission of diseases and also for implementing effective vector control measures. With the aim to understand how vector competence is controlled and to inform vector control strategies, my group focuses on the interplays between reproductive physiology and immunity in mosquito females, using as a model the mosquito *Aedes aegypti*, vector of arboviruses. The data we obtained reveal that blood feeding and mating influence arboviral immunity in mosquito females in a tissue-specific manner. Our analyses uncover important links between the female physiology and its immunity, especially in the midgut, which can affect mosquito vector competence and thus pathogen transmission. I will also present the last progress we made to identify the factors and molecular mechanisms involved in these interplays, which we will ultimately manipulate to create resistant mosquitoes.

Keywords: Mosquito, arbovirus, reproductive physiology, gut antiviral immunity, blood feeding, mating

CO358

NUTRITIONAL IMMUNOLOGY USING INSECT MODELS

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Nutrition is a key aspect of individuals' biology. While there is a growing interest on the role of nutrition in lifespan and reproduction, the implications of nutrient choice and intake to infection remain largely unexplored. This is unfortunate because in nature, individuals are exposed to a wide variety of infection challenges that can compromise their fitness and redirect the evolutionary trajectories of populations. Here, we harnessed the power of the flies *Drosophila melanogaster* and *Bactrocera tryoni* to investigate the nutritional choices of flies exposed to bacterial infections, and the implications of these choices for flies' survival after infection. Infected flies shift the protein-to-carbohydrate (P:C) ratio of their diet from a low-protein-high-carbohydrate diet. Strikingly, a low-protein-high-carbohydrate diet significantly increases the post-infection survival of flies, suggesting that the shift in P:C ratio is a way of self-medication that has strong survival significance. Interestingly, we showed that the protein-to-carbohydrate ratio in the diet is also negatively associated with the expression of antimicrobial peptides, providing insights on the molecular mechanisms underlying the

dietary shift of infected flies. In parallel, we explored other factors that might be involved in diet choices such as the composition of the gut bacteria. Increasingly understood to be vital for wellbeing, gut bacteria are known to affect a range of physiological traits including immunity and nutrition. We investigated how gut bacteria influence foraging behaviour by manipulating the type and timing of bacteria individual flies were exposed to, and examined their olfactory-guided preferences to food bacteria and nutrients. Together, our findings provide a better understanding of the relationship between diet, infection and the composition of the gut bacteria, and reveal an important foraging pattern whereby flies make use of their dietary choices to enhance their survival when facing an immune challenge.

Keywords: Nutrition, Infection, Immunity, gut bacteria

Host Regulation by Parasites and Pathogens

CO340

WOLBACHIA SURFACE PROTEIN EXPRESSION IN *ANOPHELES GAMBIAE* IS ABLE TO ELICIT THE MOSQUITO IMMUNE SYSTEM AND DECREASE THE *P. BERGHEI* INFECTION

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New tools for malaria control are needed. An innovative strategy currently being considered is the use and release of engineered mosquito strains that confer refractoriness to disease transmission. This type of engineering also holds vast potential to reveal new details on the biology of mosquito-parasite interactions. Towards this goal, we have recently generated transgenic *An. gambiae* lines, expressing the Wolbachia Surface Protein (WSP), which is known as a major surface antigen of endosymbiont Wolbachia. This protein has been previously shown to elicit innate immune induction via TLR2 and TLR4 activation in both humans and mice and to inhibit apoptosis in neutrophils through inhibition of caspase-3 activity. In mosquito cells WSP induces strong innate immune responses up-regulating antimicrobial peptide (AMP) and other immune effector genes. We expressed the WSP under the control of the blood-meal inducible vitellogenin promoter found that the transgene expression results in reduced *P. berghei* infection in one of our independent transgenic strains. We observed a significant reduction in the number of oocysts per midgut and similarly in the number of sporozoites in the salivary glands. To begin exploring the perturbation mechanism we have analysed the expression profiles of known immune-genes in both the TOLL and IMD pathways and found that several genes are differentially expressed as a result of WSP protein presence. These preliminary data have highlighted the potential to artificially reduce malaria infection in mosquitoes using single Wolbachia-derived antigens, instead of the entire organism, and may help unravel the resistance mechanism underlying the field application of these symbionts.

Keywords: Malaria, Wolbachia surface protein, Anopheles, Immune system, P. berghei

CO359

REGULATION OF BUMBLEBEES BY THEIR PARASITES: FROM INDIVIDUALS TO POPULATIONS?

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Theory tells us that parasites can regulate host populations, but we are still far from understanding how and when such regulation occurs in natural systems. Impacts on individuals, which must underlie any population regulation, are much more clearly understood. However, much of this understanding comes from focusing on single host-parasite interactions, whereas under natural conditions these interactions are embedded in complex multi-species host-parasite assemblages. In this talk I will use bumblebees and their parasites as a model system to ask what we know, and what we still need to discover. I will discuss the impacts of a range of parasites on individuals, colonies, and populations, how these impacts are embedded within complex host-parasite assemblages, and potential directions for future research.

Keywords: Bombus, parasites, Crithidia, Nosema, Sphaerularia, Syntretus

CO360

THE IMPORTANCE OF GENOME ARCHITECTURE FOR THE FUNCTION OF PARASITOID-ASSOCIATED SYMBIOTIC VIRUSES IN PARASITISM OF HOST INSECTS

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Some lineages of parasitoid wasps have evolved a remarkable strategy in their parasitism arsenal: they utilize symbiotic viruses to breach host defenses. The most well-known examples are Polydnaviruses (PDVs), which are found in three diverse clades of parasitoid wasps representing three unique origins. Despite their independent evolution, PDV genomes share some key characteristics. PDV genomes are integrated into the genomes of wasps, and their genome architecture has been rearranged compared to viral ancestors into two separate components: proviral segments containing virulence genes and replication genes. The virus replication machinery is not packaged into virions, thus the viruses and parasitoids are reliant on each other for reproduction. In addition to PDVs, symbiotic viruses have been documented in other parasitoid wasp species, and their recent genomic characterization has shown that these virus symbionts diverge from the PDV paradigm. The genome sequence of the entomopoxvirus found in *Diachasmimorpha longicaudata* venom glands (DlEPV) revealed that this virus is non-integrated and can replicate in host insects. Although the DlEPV genome differs in architecture relative to PDVs, viral transcriptome analysis shows that virulence and replication genes are partitioned in wasps and hosts at the level of expression, functionally behaving in a PDV-like manner. We have discovered another independently-derived viral symbiont in *Fopius arisanus* that has a wasp-integrated genome that produces virus-like particles (VLPs) that do not contain nucleic acids. These data highlight existing diversity in viral symbiont genome architecture, which has important implications for symbiont function in hosts. PDVs lack the replication genes to produce more virions in hosts and must rely upon gene expression from existing DNAs for virulence. DlEPV replicates in hosts, where it emphasizes virulence gene expression. Finally, the lack of DNAs in *F. arisanus* VLPs prevents delivery of virulence genes into host cells, necessitating other mechanisms for promoting parasitism in host insects.

Keywords: Symbiosis, parasitoid, virus

CO361

GENOMIC INSIGHTS INTO STRATEGIES USED BY DIFFERENT PARASITOID WASPS

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Parasitoid wasps comprise one of the most species-rich groups of animals on earth. Many parasitoid wasps are also used to control pest species of worldwide importance to humans. Parasitoids successfully parasitize hosts by producing virulence factors from sources that include venom, teratocytes and symbiotic polydnaviruses. However, no studies have accessed genome-wide analyses of strategies used by different parasitoid wasps. We sequenced and analyzed the genomes of three endoparasitoid wasps (*Cotesia vestalis*, *Diadegma semiclausum*, and *Diadromus collaris*) that parasitize the diamondback moth *Plutella xylostella*. Results identified the inventory of genes encoded by each species. Sites of integration for the symbiotic polydnaviruses carried by *C. vestalis* and *D. semiclausum* were also identified. *C. vestalis*, *D. semiclausum*, and *D. collaris* produce some putative virulence factors that are similar to one another but the polydnaviruses associated with *C. vestalis* and *D. semiclausum* largely encode different genes while the venoms of *C. vestalis* and *D. collaris* also largely differ.

Keywords: Parasitoid, host, genome, transcriptome, proteome

CO362

USING OF WAX MOTH *GALLERIA MELLONELLA* AS A MODEL SPECIES FOR STUDYING OF MICROEVOLUTIONARY RELATIONSHIP BETWEEN HOST AND PARASITE

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The greater wax moth (*Galleria mellonella* (L.)) is considered a pest of beehives and a common model species used for immune and physiological research, as well as for testing infections of insects and mammals. This species is practical for rearing in laboratory conditions and become popular to study evolutionary processes in different host-parasite systems. Our work is focused on using wax moth as a model to uncover microevolutionary mechanisms development of insects resistance for entomopathogenic bacteria *Bacillus thuringiensis* and fungi Beauveria and Metarhizium. We found that insects selected for resistance, develop unique adaptations of immune system against different groups of parasites. This was related with specificity of infections processes and life strategies, toxins and enzymes of pathogens. The results show that during the microevolution, epigenetic mechanisms (DNA methylation, histone acetylation and microRNA) are involved in the formation of insect resistance to entomopathogenic microorganisms. Analysing the main trends in the evolution of insects resistance to fungal and bacterial pathogens, it can be concluded that the development of insect resistance is due to the complex enhancement of immunological and physiological defense reactions at the site of the pathogens penetration in the host. Thus, one of the microevolutionary mechanisms of insect resistance to insect pathogenic fungi and bacteria is the prioritizing and re-allocation of stress and immune components of the host to the "front line" *i.e.* integument against fungi and midgut for bacteria. *This work was supported RSF №16-14-10067.*

Keywords: Wax moth, microevolution, resistance, host-parasite coevolution, defence reactions

CO363

A MALARIA SIGNAL FOR IMMEDIATE TRANSMISSION

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Malaria infections render humans more attractive for *Anopheles gambiae* mosquitoes but the underlying molecular mechanisms is not well understood. We recently published a report (Science 2017, 355: 1076-1080) demonstrating that supplementation of the mosquito blood meal with the parasite specific isoprenoid precursor, (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) mimics the effects of parasitized blood on mosquito behavior. HMBPP triggers an increased release of CO^2, aldehydes and monoterpenes in a complex mixture that strongly enhances female mosquito attraction. Females take larger blood meals of the HMBPP supplemented blood and yet mosquito fecundity and lifespan are unaffected. Moreover, when HMBPP is administered in a *Plasmodium falciparum* infected blood meal, the number of parasites developing in the mosquito is increase. Transcriptome analyses reveal temporal up-regulation of immune genes as well as neural genes potentially involved in foraging and feeding while genes involved in oogenesis and translation are down-regulated. The importance of putative release of HMBPP not only by parasites but also gut bacteria will be discussed.

Keywords: *Plasmodium falciparum*, *Anopheles gambiae*, (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP), parasite host manipulation, volatile attraction of mosquitoes, temporal transcriptome analyses

CO364

THE DISCOVERY OF RNA VIRUSES IN GLOBAL INVASIVE SPECIES, BACTROCERA DORSALIS, IMPLYING AN INTENSIVE INTERACTION BETWEEN RNA VIRUSES AND THE HOST RNAI PATHWAY

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The oriental fruit fly (*Bactrocera dorsalis*) is an important agricultural pest in Asia, Africa, and the Pacific, attacking over 250 host plants. As an invasive pests, it has gradually expanded from tropic to sub tropic regions causing severe economic loss and trade restrictions. Species being polyphagous and invasive, encounter with more potential microbes from the species infesting on the same hosts and/or from native hosts in invaded regions. RNA viruses, as the most widely distributed viruses, are a class of viruses that use RNA as a genetic material. Insects are commonly infected with multiple viruses including those that cause sublethal, asymptomatic, and latent infections. Here, we report the discovery of nine RNA viruses with nearly complete genome from the transcriptomes of the artificial diet reared *B. dorsalis* strain (avoiding environmental viral contamination). Briefly, four belong to positive stranded RNA virus: ssRNA(+) including Cripavirus, Iflavirus, Negevirus group and unassigned Picornaviridae, two belong to negative stranded RNA virus: ssRNA(-) including unassigned Rhabdoviridae and Chuvirus group, and three belong to double stranded RNA virus: dsRNA including Orbivirus and two Toti-like viruses. The prevalence of these viruses has been detected in three geographical populations and various insect developmental stages, which showed that all the viruses were confirmed in field populations except two viruses (Picornaviridae and Orbivirus) could only be detected in insect immature stages. To have a view of these viruses impacting on the host, parallel with transcritpme sequencing, the small RNAs were also sequenced. Intriguingly, six (including all three groups ssRNA(+), ssRNA(-) and dsRNA) out of nine viruses showed a typical 21 nt bp signal of viral-derived small RNAs which suggests the activity of insect RNAi core machinery Dicer-2 in processing viral-related dsRNA as a host antiviral strategy, implying an intensive interaction of the RNA viruses and the fly RNAi pathway.

Keywords: Invasive pests, insect RNA viruses, RNAi, immunity,

CO365

A TWO-LEVEL MECHANISM OF SPECIFICITY – VENOM MOLECULES *PER SE* AND THEIR VESICULAR TRANSPORT – LIKELY DETERMINES THE DROSOPHILA HOST RANGE OF THE PARASITOID *LEPTOPILINA BOULARDI*

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Successful development of *Leptopilina boulardi* wasps inside *Drosophila melanogaster* larvae is ensured by injection of venom at oviposition which notably circumvents the host immune response. Interestingly, *L. boulardi* venom not only contains secreted proteins but also extracellular vesicles (venosomes) we have shown to be essential for parasitic success by acting as carriers of certain proteins into specific immune cells of the host, the lamellocytes. To better understand host specificity of *L. boulardi*, we conducted two types of experiments. i) An experimental evolution protocol was designed to simultaneously analyze on different Drosophila hosts the evolution of *L. boulardi* parasitic success and venom composition (semiautomatic analysis of 1D electrophoretic profiles plus specific approach using antibodies). We evidenced a rapid and differential evolution of venom protein composition in response to the host. Besides, data suggest that *L. boulardi* venom contains a combination of proteins "specialized" to each host, as well as more ""broad-spectrum"" proteins. ii) Using co-immunolocalization assays (venosomes / transported proteins), we tested whether *L. boulardi* (ISm strain) venosomes were internalized in lamellocytes of different host species. We observed a strong variation of the uptake capacity of venosomes and their quantity in lamellocytes according to the Drosophila species tested (*D. simulans*, *D. yakuba*, *D. suzukii*), which correlated with the rate of success of *L. boulardi* ISm. Venosomes act as interspecies transport vehicles involved in parasite virulence that may thus also represent a new host specificity level. Overall, *L. boulardi* likely relies on a two-level mechanism to ensure host species specificity.

Keywords: Parasitoid wasp, Drosophila, specificity, venom, venosomes, experimental evolution, lamellocytes

Session 13.
Physiology and Biochemistry

Insect Development

CO366

HOW DO MOTHER'S TRANSFER PHOTOPERIODIC INFORMATION TO THEIR PROGENY?

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One of the fascinating aspects of insect diapause is that in many cases the mother receives photoperiodic information that is conveyed by unknown mechanisms to her progeny, thereby determining their diapause fate. How is such information transferred between generations? In flesh flies, changes in histone acetylation and microRNAs are among the candidates that appear to be involved in a maternal effect that shuts off the diapause response in pupae of the next generation. Acetylation profiles are specific for diapausing and nondiapausing pupae, and select microRNAs are significantly overexpressed in such nondiapausing pupae and may prevent diapause entry, thus suggesting important roles for these new players in regulating diapause.

Keywords: Diapause, maternal effects, microRNAs, epigenetics

CO367

THE INTRIGUING ROLE OF BRACOVIRUS ANKYRIN PROTEINS IN AFFECTING INSECT DEVELOPMENT

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The ankyrin repeat is a protein-protein interaction motif found in many proteins. The wide variety of molecules with which the ankyrin domain proteins (ANK) interact is reflected at the various cellular processes in which these proteins are involved. Polydnaviruses (Polydnaviridae - PDVs) are obligate symbionts of some wasps that parasitize lepidopteran larvae; those associated with braconid wasps belong to the genus *Bracovirus* (BV). PDV free viral particles, which are injected into the host at oviposition, express virulence factors that impair host immunity and development. Many PDVs encode for proteins characterized by ankyrin repeats. These ANK proteins are similar to insect and mammalian IκB, but the lack of regulatory domains for signal-mediated degradation and turnover confers them an immunosuppressive activity. We used *Drosophila melanogaster* as a model to carry out a functional analysis of ANK proteins encoded by the bracovirus associated with the wasp Toxoneuron nigriceps (TnBV), which is a larval parasitoid of the noctuid moth *Heliothis virescens*. We analyzed the effect on Drosophila development of the expression of two ank genes, TnBVank1 and TnBVank3. Drosophila larval developmental transitions occur via a series of pulses of the hormone ecdysone, which is synthetized in the prothoracic gland (PG). We found that either TnBVank1 or TnBVank3 expression in the Drosophila PG block the larval-pupal molt. This phenotype mimics the developmental arrest of parasitized *H. virescens larvae*. These two viral genes disrupt ecdysone biosynthesis by exerting synergistic and non-overlapping functions. TnBVANK1 impairs the vesicular traffic of ecdysteroid precursors, while TnBVANK3 affects the expression of steroidogenic genes. Our findings expand our current knowledge of host-parasitoid interactions, providing new insights on the pleiotropic functions of ANK genes.

Keywords: Development, Drosophila, bracovirus, ANK proteins, Ecdysone biosynthesis,

CO368

MULTIPLE MIRNAS JOINTLY REGULATE THE BIOSYNTHESIS OF ECDYSTEROID IN THE HOLOMETABOLOUS INSECTS, *CHILO SUPPRESSALIS*

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The accurate rise and fall of active hormones is important for insect development. The ecdysteroids must be cleared in a timely manner. However, the mechanism of suppressing the ecdysteroid biosynthesis at the right time remains unclear. Here, we sequenced a small RNA library of *Chilo suppressalis* and identified 300 miRNAs in this notorious rice insect pest. Microarray analysis yielded 54 differentially expressed miRNAs during metamorphosis development. Target prediction and *in vitro* dual luciferase assays confirmed that seven miRNAs (two conserved and five novel miRNAs) jointly targeted three Halloween genes in the ecdysteroid biosynthesis pathway. Overexpression of these seven miRNAs reduced the titer of 20-hydroxyecdysone (20E), induced mortality, and retarded development, which could be rescued by treatment with 20E. Comparative analysis indicated that the miRNA regulation of metamorphosis development is a conserved process but that the miRNAs involved are highly divergent. In all, we present evidence that both conserved and lineage-specific miRNAs have crucial roles in regulating development in insects by controlling ecdysteroid biosynthesis, which is important for ensuring developmental convergence and evolutionary diversity.

Keywords: Insect, miRNA, metamorphosis, development, ecdysteroid

CO369

DIVERSE MOLECULAR MECHANISMS CONTROL GLIAL PHAGOCYTOSIS DURING CNS DEVELOPMENT OF *DROSOPHILA MELANOGASTER*

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Central Nervous System (CNS) development is accompanied by massive neuronal apoptosis. In the CNS of *Drosophila melanogaster* about 50% of neurons die during different developmental stages, mostly at late embryogenesis and early metamorphosis, and are efficiently removed by neighboring phagocytic glia. Glial cells perform their phagocytic function through precise recognition, engulfment and degradation of dying neurons. Two phagocytic receptors, Six-Microns-Under (SIMU) and Draper (Drpr) play a crucial role in Drosophila glial phagocytosis. We have previously shown that loss of simu or draper leads to impaired removal of apoptotic neurons during embryogenesis. Our recent study demonstrates that phagocytosis of apoptotic neurons during metamorphosis employs different molecular mechanisms. We show that SIMU is not involved in glial phagocytosis at metamorphosis, but it is mostly expressed in pupal macrophages outside the brain. Our detailed analysis of Drpr function in different glial subtypes shows that during metamorphosis Drpr is required for phagocytosis of apoptotic cells by ensheathing glia and astrocytes but not by cortex glia. Most importantly, we demonstrate that unlike in the embryonic CNS, in pupal brain Drpr activates c-Jun N-terminal kinase (JNK) signaling, which is required for efficient removal of apoptotic neurons. We also found that Drpr may trigger an additional signaling pathway, other than JNK that is capable of removing apoptotic neurons in the pupal brain. Our data demonstrate that studying glial phagocytosis in the evolving CNS at distinct developmental stages is critical for characterizing the diverse molecular mechanisms and cell types that temporally and spatially regulate this convoluted process.

Keywords: Drosophila, glia, phagocytosis, development, CNS, SIMU, Drpr, JNK

CO370

ANTAGONISTIC ACTIONS OF JUVENILE HORMONE AND 20-HYDROXYECDYSONE WITHIN THE RING GLAND DETERMINE DEVELOPMENTAL TRANSITIONS IN DROSOPHILA

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In both vertebrates and insects, developmental transition from the juvenile stage to adulthood is regulated by steroid hormones. In insects, the steroid hormone, 20-hydroxyecdysone (20E), elicits metamorphosis, thus promoting this transition, while the sesquiterpenoid juvenile hormone (JH) antagonizes 20E signaling to prevent precocious metamorphosis during the larval stages. However, not much is known about the mechanisms involved in crosstalk between these two hormones. In this study, we discovered that in the ring gland (RG) of Drosophila larvae, JH and 20E control each other's biosynthesis. JH induces expression of a Krüppel-like transcription factor gene Kr-h1 in the prothoracic gland (PG), a portion of the RG that produces the 20E precursor ecdysone. By reducing both steroidogenesis autoregulation and PG size, high levels of Kr-h1 in the PG inhibit ecdysteriod biosynthesis, thus maintaining juvenile status. JH biosynthesis is prevented by 20E in the corpus allatum, the other portion of the RG that produces JH, to ensure the occurrence of metamorphosis. Hence, antagonistic actions of JH and 20E within the RG determine developmental transitions in Drosophila. Our study proposes a mechanism of cross-talk between the two major hormones in the regulation of insect metamorphosis.

CO371

MOSQUITOES REQUIRE GUT MICROBES FOR DEVELOPMENT

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Mosquitoes host communities of microbes in their digestive tract that consist primarily of bacteria. Several mosquito species including *Aedes aegypti* do not develop beyond the first instar in the absence of a gut microbiota. In contrast, several species of bacteria, including *Escherichia coli*, rescue development of axenic larvae into adults. The molecular mechanisms underlying bacteria-dependent growth in contrast are unknown. Here we designed a genetic screen around *E. coli* that identified high affinity cytochrome bd oxidase as an essential bacterial gene product for mosquito development. Bioassays showed that bacteria in non-sterile larvae and gnotobiotic larvae inoculated with wild-type *E. coli* reduced midgut oxygen levels below 5%, whereas larvae inoculated with *E. coli* mutants defective for cytochrome bd oxidase did not. Experiments further supported that hypoxia stimulates growth through activation of hypoxia inducible transcription factors. Altogether, our results identify aerobic respiration by bacteria and other members of the gut microbiota in mosquito development.

Keywords: Microbiota, growth, stem cell, midgut, metabolism

CO372

THE REMODELING OF LARVAL ORGANS IN LEPIDOPTERA DURING METAMORPHOSIS: THE END OF AUTOPHAGIC CELL DEATH?

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Metamorphosis represents a critical phase in the development of holometabolous insects. During the transition from the larval to the adult phase, several organs are remodeled or disappear as a result of apoptosis and autophagy. While apoptosis brings about cell death, the role of autophagy in this biological setting is quite controversial. In particular, although numerous studies have analyzed this self-eating process in Lepidoptera, none of them has demonstrated a causative role of autophagy in cell death in larval organs. In recent years we focused attention on the degeneration of the larval midgut and posterior silk gland. Our data demonstrate that in the silk gland autophagy is necessary to produce the energy the gland cells need to stay alive during the spinning process of the cocoon, while in the midgut the setting is more complex and autophagy acts in a coordinated manner with apoptosis. In fact, while autophagy starts when the larva ceases to feed and permits larval midgut cells to survive starvation during early metamorphosis, and allows for the catabolic degradation of the cell components that can be used by the newly forming pupal-adult epithelium, apoptosis intervenes later and actually drives the demise of the larval epithelium. In conclusion, according to the evidence collected in both organs, autophagy plays a pro-survival rather than a pro-death role in the larval organs of Lepidoptera during metamorphosis.

Keywords: Apoptosis, autophagy, *Bombyx mori*, metamorphosis, midgut, silk gland

Sensory System

CO373

SOLUBLE OLFACTORY PROTEINS IN INSECTS

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Insect olfactory neurons are bathed in a chemosensillar lymph containing small soluble proteins acting as carriers for odorants and pheromones. Different families of these proteins have been described; Odorant Binding proteins (OBPs), the first to be discovered and the best studied, are 120-150 amino acid long and present a compact structure made of six α-helical domains, while Chemosensory proteins (CSPs), also made in α-helical segments are smaller (110-130 amino acids) and folded in a different fashion. Structural studies and functional characterization have shown that members of both families bind general odorants or pheromones and different pieces of evidences indicate that OBPs are important for a correct detection of chemical stimuli. A third class of insect olfactory carriers, NPC2 (Niemann-Pick C2), have an eight β-sheets structure. Although they have been known for a long time in vertebrates as cholesterol carriers, NPC2 proteins were only recently proposed as semiochemical carriers, after some members have been found in high concentrations in the antennae of different species of insects and more recently in olfactory organs of some Arachnida. OBP genes are only present in Hexapoda, but structurally similar proteins named OBP-like, have recently been reported in some Acari. On the contrary, CSPs have been reported only in Crustacea, and Hexapoda, while NPC2 proteins seem to be present in the whole animal kingdom, although limited information is available. For all these protein families, several members have also been identified in exocrine glands, where they may be involved in the release of semiochemicals, but other functions may be hypothesized due to their presence in tissues lacking evident function in chemical communication.

Keywords: Chemical communication, Odorant Binding proteins, Chemosensory proteins, Niemann-Pick C2, OBP-like proteins

CO374

INSECT OLFACTION: A SOURCE OF INSPIRATION FOR THE DEVELOPMENT OF NEW BIOSENSORS

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Insects use chemical perception to interact with other insects and with the environment. The perception of volatile substances, linked to the processes of feeding, mating and escape from predators, is related to gene families encoding for proteins belonging to Odorant Binding Proteins (OBPs), Olfactory Receptors (ORs), Ionotropic Receptors (IRs) and Chemosensory Proteins (CSPs). The study of insects physiology at the molecular level and the implementation of innovative technologies, allow the development of applications able to cope with many human issues, through the use of biotechnology. The innovative aspect consists in the ability to exploit natural and physiological phenomena, as a source of inspiration for biotechnological applications, useful to improve life quality. The success of OBPs in nature, highlighted by the adaption of these proteins to a large number of diverse tasks, attracted the attention of scientists interested in the development of biosensors. Specific OBPs of scavenger insects, able to perceive volatiles indicative of decomposition processes, could be used for the construction of biosensors able to monitor decomposition processes in foods, in order to ensure the quality, safety and food shelf life. Moreover, different volatile molecules, such as terpenes and esters are related to wine quality, typicity or alteration. These same molecules are similar or in some cases identical to pheromones produced and perceived by some species of insects. These insect OBPs can be used as the biosensing element for the construction of artificial devices for the monitoring of wines quality.

Keywords: Odorant Binding Proteins, biosensor, volatile molecules, biomimetics

CO375

ODOR EVOKED ACTIVITY ACROSS BRAIN LAYERS: CODES, PATTERNS AND MEMORIES

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Most odors are encoded by combinatorial activity patterns across neurons, in sequential neural layers: olfactory receptor neurons (vertebrate nose, insect antennae), olfactory glomeruli (olfactory bulb, antennal lobe), then higher order brain centers (piriform cortex, mushroom body). Olfactory stimuli elicit complex spatio-temporal patterns of neural activity. This raises the question about the readout of the code: does the brain need the full spatio-temporal pattern to recognize an odor, or is a snapshot sufficient? Learning experiments show that odors are recognized almost immediately at stimulus onset, also when during training they are learned later or even after stimulus offset. This suggests that different time windows fulfil different tasks, or are used in different ways by the brain. We have therefore analyzed the temporal development of odor information in different olfactory neuron populations in the fruit fly *Drosophila melanogaster*: olfactory receptor neurons, projection neurons in the antennal lobes (dendrites and somata), and Kenyon Cells in the mushroom bodies (dendrites and somata). We found that, when measuring calcium concentration changes, odor information was stable during odorant presentation both for short and long stimuli, and changed after odor offset, providing reliable information about both odorant onset and offset. For a subset of Kenyon cell somata, however, we found that odor information was maintained for several seconds beyond odor offset, suggesting a neuronal substrate for sensory memory that could be used in trace conditioning situations, *i.e.* when a reward arrives after stimulus offset. Our data also show that in order to understand information across neural networks it is necessary to analyze subcellular compartments: information across cell bodies has different temporal progression (and information content) than across dendrites, possibly indicating within cell multitasking.

Keywords: Olfaction, odor coding, antennal lobe mushroom body

CO376

ECOLOGICALLY LABELED LINES IN THE INSECT OLFACTORY SYSTEM

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Insect behavior is to a great extent smell-guided in most fitness related situations; for optimal reproduction and survival. We are studying the olfactory functions underlying these behaviors in natural and semi-natural settings. By combining a host of methods we have established the presence of specific neural lines, stretching all the way from sensory neurons on the antenna and palp, up to higher brain centers. When such a line is activated, the ensuing behavior can be predicted with high certainty. Such lines have obviously been favored by evolution to provide safe and fast information processing regarding specifically important odor cues. They do, however, also open up for exploitation by other organisms as parasites and predators. In drosophilid flies we have established the function of a number of these lines involved in partner location, food search, and pathogen and parasitoid avoidance. By using modern opto- and thermogenetic tools we have been able to show how specific behaviors can be triggered by artificial activation. We could also show how pathogens use olfactory lines involved in sexual behavior for their own propagation. In sphingid moths we have established specific lines involved in partner and food search, and in location of suitable oviposition sites. We have also discovered a new olfactory organ at the tip of the proboscis, an organ which is crucial for nectar feeding from a preferred flower species. Here, we can only predict the existence of labeled lines as we still lack the neurogenetic tools. With Crispr-Cas9 techniques we are presently modifying moths genetically to make such manipulations possible.

Keywords: Olfaction, smell, behaviour, drosophila, manduca, neuroethology

CO377

STOPPING THE CIRCADIAN CLOCK: AN EFFECTIVE WAY TO ADJUST PHASE IN THE GERMAN COCKROACH

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To adjust circadian phase, requires several time consuming steps to know the exact phase of the individual and apply an effective light pulse. If the circadian clock is stopped, the phase can be shifted to the desired time by waiting the time-lag passed. The German cockroach will go into chill coma condition when it is exposed at 4°C. It can survive under chill coma condition for at least 24 hours. But, the mortality increases along with the exposure time after 24 hours. Under chill coma for 24, 48 or 72 hours, it does not cause damage on DNA even though the mortality does occur after 24 hours. The clock genes remain static without fluctuating under chill coma condition until the cockroach is revived at 28°C. The expression of clock genes continues its fluctuation after waking up from chill coma. The circadian rhythm of clock genes expression is reflecting on the locomotor pattern of male cockroaches with 12 hours blank (no activity) for 12 hours at 4°C. Based on the results, the circadian clock of the German cockroach can be stopped under 4°C condition.

Keywords: *Blattella germanica*, circadian phase, clock genes, locomotion

CO378

THE ENERGY CONSUMPTION AND PERFORMANCE OF FLY PHOTORECEPTORS

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The contribution of voltage-dependent conductances to the energy efficiency of coding by graded potentials in photoreceptors remains unclear. Two voltage-dependent delayed rectifier K+ conductances (DRs) shape the voltage response of blowfly photoreceptors and contribute to their light adaptation. By investigating the energy efficiency of analogue coding of blowfly R1-6 photoreceptors using modelling, we resolved the function of their two main voltage-dependent conductances. Broadly, they make two types of energy saving. Firstly, by reducing membrane resistance upon depolarization they convert the cheap, low bandwidth membrane needed in dim light to the expensive high bandwidth membrane needed in bright light. This investment of energy in bandwidth according to functional requirements can halve daily energy consumption. Secondly, negative feedback produced by DRs reduces membrane impedance and increases bandwidth. This negative feedback allows an active membrane with DRs to consume at least 30% less energy than a passive membrane with the same capacitance and bandwidth. Voltage-dependent conductances in other non-spiking neurons, and in dendrites, might be organized to make similar savings.

Keywords: Energy consumption, information processing, ion channel, vision, blowfly

CO379

THE SPIKE BURSTS OF THE ANTENNAL THERMO– AND BIMODAL HYGROTHERMORECEPTOR NEURONS PROBABLY INDUCE ESCAPE BEHAVIOUR IN INSECTS TO AVOID OVERHEATING

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From all the abiotic factors influencing insects daily, temperature is undeniably most important one. Lethal temperatures for insects are not extremely high, body temperatures around 45°C kills. Since they regulate their body temperature behaviourally, it is important to make smart decisions instantaneously, because due to the low body mass, time to reach critical body temperature lays in seconds. Insects use thermosensory sensilla on the antennae to detect noxious high temperatures. Since mechanism behind this process of ground dwelling insects are scarcely studied, we focused our research on them – carabid beetle, *Pterostichus oblongopunctatus* (Coleoptera: Carabidae) and click beetle, *Agriotes obscurus* (Coleoptera: Elateridae). Both of these species encounter dangerously high temperatures on summer when the temperature of soil surface, in direct sunlight, reaches 50°C. The antennal dome-shaped sensilla (DSS), inhabited by thermoreceptor neuron and two hygrothermoreceptor neurons, was used for measurements of the neuronal responses; locomotion was measured to describe changes in behaviour. Both experiments were done in various temperature conditions that were raised up to 45 degrees. On both insect groups hygroreceptor neurons started to react to temperature when preference temperature zone was breached. Even more, all the neurons in DSS started to produce high temperature induced spike bursts. The threshold temperatures at which they switched from regular firing to spike bursting differs, but it covers the entire noxious high temperature zone starting from the end of preference temperature. Also noticeable is that the bursts are temperature dependent and increase with temperature increase. Behavioural studies showed that on noxious high temperature zone locomotor activity was elevated. The threshold temperatures for bursting and elevated mobility of the beetles coincided, therefore indicating that the spike bursts of the antennal thermo– and bimodal hygrothermoreceptor neurons are responsible for detecting noxious high temperatures to avoid overheating and induce escape behaviour in these insects.

Keywords: Dome-shaped sensillum, Moist-hot neuron, Dry-hot neuron, Cold-hot neuron

CO380

TASTE RECEPTOR PLASTICITY IS RELATED TO FEEDING HISTORY IN TWO CONGENERIC SPECIES OF PAPILIONIDAE (LEPIDOPTERA)

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The responsiveness of gustatory receptor neurons (GRNs) in insects depends on various factors, such as larval instar, feeding history, physiological state and time of day. The GRN spike activity of the maxillary lateral and medial taste chemosensilla in the larvae of two related species of Lepidoptera (*Papilio machaon* L. and *Papilio hospiton* Géné) raised on different host plants, was electrophysiologically recorded after stimulation with sugars, bitters and NaCl as well as host plant saps, with the aim of cross-comparing their response patterns and evaluating any effects of different feeding histories. For this purpose the larvae were raised on their preferred host plant (*Foeniculum vulgare* for *P. machaon* and *Ferula communis* for *P. hospiton*). In addition, *P. machaon* larvae were also raised on Ferula communis. The taste discrimination capabilities and modalities of the two species were measured and cross-compared to evaluate convergence and/or divergence phenomena linked to the insect feeding history. The results show

that: a) the GRN responsiveness of both sensilla in *P. machaon* raised on *Fe. communis* differs significantly from that of *P. machaon* on *Fo. vulgare*, but is not different from *P. hospiton* on *Fe. communis*; b) *P. machaon* larvae raised on *Fe. communis* exhibit response spectra somewhat intermediate between those of *P. machaon* on fennel and of *P. hospiton* on *Fe. communis*, the latter two showing a wider difference from each other; c) the pattern of GRN activity generated by each plant sap in both sensilla of *P. machaon* raised on *Fe. communis* is different from that generated when raised on *Fo. vulgare*, but no difference was found with *P. hospiton*. The data support the assumption that diet-related factors may influence peripheral chemosensitivity in lepidopterous larvae.

Keywords: Feeding history, taste plasticity, discrimination, Papilionidae

CO381

ANTENNAL TRANSCRIPTOME ANALYSIS AND EXPRESSION PROFILES OF ODORANT BINDING PROTEINS IN *STRELTZOVIELLA INSULARIS* (LEPIDOPTERA: COSSIDAE)

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Streltzoviella insularis (Staudinger) is a destructive boring pest in China, mainly damaging *Fraxinus pennsylvanica*, *Sophora japonica*, *Ginkgo biloba*, and the male *S. insularis* moth is high attracted by the sex pheromones released by the female moth. However, the molecular mechanism of how sex pheromones are perceived by male *S. insularis* moth are still rudimentary. In this study, we established the antennal transcriptome of *S. insularis*, we identified 32 putative odorant-binding proteins (OBPs), 15 putative chemosensory proteins (CSPs), 41 odorant receptors (ORs), 11 gustatory receptors (GRs), 11 ionotropic receptors (IRs), and 2 sensory neuron membrane proteins (SNMPs). The differential expression profiles of odorant-binding proteins (OBPs) are evaluated by fluorescence quantitative real-time PCR. These results establish and improve the basis knowledge of the olfactory receptive system, furthermore provide a theoretical basis for the development of new pest control method.

Keywords: *Streltzoviella insularis*, Olfactory proteins, Expression profile

Insect Metabolism and Nutrition

CO382

INSECT NUTRITION: WHY BALANCE MATTERS

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All animals, including insects, eat to acquire multiple essential nutrients that fuel the processes of growth and reproduction. However, the foods that animals eat rarely contain nutrients in optimal amounts or blends. Thus, the challenge for animals, including insects, is to eat a combination of foods that provides a balance of nutrients that optimizes performance. This talk starts by outlining which nutrients are generally important for insects. It then reviews an experimental framework – nutritional geometry – that is being used to study interactions between multiple nutrients. Next, it draws on examples from grasshoppers and caterpillars to show how variation in two key nutrients – proteins and digestible carbohydrates – influence growth and reproduction, including the degree of nutritional heterogeneity these insect herbivores can experience under natural conditions. It concludes by reviewing experiments, using fruit flies, that reveal molecular responses to variation in food protein-carbohydrate content.

Keywords: Nutritional Geometry, Protein, Carbohydrates, Nutrient Landscape, Herbivores

CO383

IDENTIFICATION OF AN ADIPOKINETIC HORMONE-RESPONSIVE TRIACYLGLYCEROL LIPASE IN *DROSOPHILA MELANOGASTER*

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Adipokinetic hormone (Akh) is a key regulator of storage fat mobilization in a variety of insect species including the vinegar fly *Drosophila melanogaster*. Previously, we and others provided *in vivo* evidence that Drosophila flies mutant for the Akh neuropeptide or the Akh receptor suffer from defects in storage lipid homeostasis. Under *ad libitum* feeding conditions Akh signaling mutants accumulate excessive body fat stores but are incapable to fully mobilize them in response to starvation. Importantly, genetic data support a dual control of starvation-induced fat mobilization by the complementary actions of the evolutionarily conserved triglyceride lipase Brummer and of the Akh-signaling pathway. However, to date *in vivo* support for the identity of any Akh-responsive lipase(s) in Drosophila is missing. To identify Akh-dependent lipase(s) in Drosophila we performed a sensitized *in vivo* RNA interference screen targeting annotated lipase genes to identify the Phosphatidic Acid Phosopholipase A1 (*PAPLA1*) gene as the primary candidate. Notably, *PAPLA1* is a Drosophila homolog of the Manduca sexta Triglyceride lipase, which has been implicated in the Akh-responsive fat mobilization of the tobacco hornworm. Surprisingly, *PAPLA1* knock out flies do not display the lipid metabolism phenotypes characteristic for mutants of the Akh signaling pathway. However, double mutant analysis with Brummer lipase and the enzymatic characterization of *PAPLA1* support the conclusion that this lipase contributes to the Akh-dependent storage fat mobilization in Drosophila.

Keywords: Drosophila, Adipokinetic hormone, triacylglycerol lipase, storage lipid mobilization, *PAPLA1*

CO384

INSULIN-LIKE SIGNALLING IN *RHODNIUS PROLIXUS*, THE VECTOR OF CHAGAS DISEASE

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Insulin-like peptides (ILPs) are functional analogs of insulin and have been identified in many insect species. The insulin/insulin-like growth factor (IGF) pathway is a conserved coordinator of growth, development and metabolism of lipids and carbohydrates. In the present study we have identified one ILP, one IGF and one insulin receptor (IR) in the blood-gorging bug, *Rhodnius prolixus*, and investigated their downstream signalling pathways. Expression profile analyses showed that the Rhopr-ILP transcript is predominantly present in the brain, whilst the Rhopr-IGF transcript is distributed in a variety of tissues; fat body, dorsal vessel and the central nervous system (CNS). A partial transcript for the Rhopr-IR (a tyrosine kinase receptor) has been cloned and found to be expressed in all tissues tested, with the highest values observed in the CNS. BpV(phen) (an IR activator), glucose, or porcine insulin each activated protein kinase Akt, an evolutionarily conserved key regulator of the intracellular insulin signalling cascade, in *R. prolixus* fat body. We also observed phosphorylation of Akt and its downstream targets glycogen synthase kinase 3 (GSK3) and transcription factor FOXO1 following a blood meal. These data suggest that endogenous Rhopr-ILP operates on an IR-like tyrosine kinase receptor. Using dsRNA, we have knocked-down the expression of either Rhopr-ILP, Rhopr-IGF, or Rhopr-IR transcripts and examined effects on metabolism and growth. Knocking down the Rhope-ILP or Rhopr-IR transcripts increased the levels of lipids and carbohydrates in the haemolymph, whilst altering lipid and carbohydrate storage in the fat body and skeletal muscle. Knock-down of the Rhopr-IGF transcript resulted in defective growth and development of fifth instars into adults, with insects displaying abnormal morphological features such as smaller wings and reduced body size.

Keywords: Neuroendocrinology; Neurohormones; Signalling pathways; dsRNA; Insulin; IGF

CO385

NUTRITIONAL REGULATION OF JUVENILE HORMONE SYNTHESIS IN MOSQUITOES: A METABOLIC ANALYSIS

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Juvenile hormone (JH) regulates reproductive maturation in insects; therefore interruption of JH biosynthesis has been considered as a strategy for the development of target-specific insecticides. The corpora allata gland (CA) from mosquitoes is highly specialized to supply variable levels of JH, which are linked to ovarian maturation and influenced by developmental and nutritional signals. However, little is known about how JH synthesis regulation is translated into changes in the CA machinery. Our studies are providing a glance into the metabolomics of the mevalonic acid (MVA) and juvenile hormone synthesis pathways in the CA of insects. Our results revealed dynamic changes in JH synthesis and exposed part of a complex mechanism that regulates CA activity. Both pathways (MVAP and JH-branch) are transcriptionally co-regulated as a single unit, and catalytic activities for the enzymes of the MVAP and JH-branch changed in a coordinate fashion. Metabolite studies showed that global fluctuations in the intermediate pool sizes in the MVAP and JH-branch were often inversely related. The metabolite changes suggest that in female mosquitoes, there are at least 4 developmental switches that alter JH synthesis by modulating the metabolic flux at distinctive points in both pathways. The fine-tuning of JH synthesis to different physiological stages in mosquito reproduction occurs through changes in the size of precursor pools, rather than by changes in mRNA and enzyme capacity levels. In addition, we applied two different quantitative approaches that using experimental data on enzymatic activities and/or metabolites concentrations modeled variations in the flux of metabolites in the JH pathway. Generalized additive models (GAMs) are regression models used to describe non-linear relationship between response and explanatory variable(s). Ordinary differential equations (ODEs) are mathematical models that allows us to gain insight on the temporal behavior of metabolites concentrations in the JH pathway using enzymatic activities as parameters.

Keywords: Mosquito, juvenile hormone, nutrition, metabolic, reproduction

CO386

THE INVOLVEMENT OF RHOPR-CRF/DH IN FEEDING AND REPRODUCTION IN THE BLOOD-GORGING BUG, *RHODNIUS PROLIXUS*

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Rhodnius prolixus is a blood-gorging insect and a vector for human Chagas disease. The insect transmits the disease following feeding, when it excretes urine and feces contaminated with the *Trypanosoma cruzi* parasite. A corticotropin-releasing factor-related peptide acts as a diuretic hormone in *R. prolixus* (Rhopr-CRF/DH); however, its distribution throughout the insect's central nervous system (CNS) and expression of its receptor (Rhopr-CRF/DHR) in other feeding-related tissue as well as the female reproductive system suggests a multifaceted role for the hormone beyond that of diuresis. Here we investigate the involvement of Rhopr-CRF/DH in feeding and reproduction in *R. prolixus.* Immunohistochemistry of the CNS showed diminished CRF/DH-like staining in neurosecretory cells of the brain and mesothoracic ganglionic mass immediately following feeding, and partial restocking of those same cells two hours later. Rhopr-CRF/DH is present in the haemolymph during diuresis, and acts synergistically with serotonin to stimulate the rapid post-prandial diuresis. Artificially elevating haemolymph Rhopr-CRF/DH titres by injection of Rhopr-CRF/DH 2 h prior to feeding resulted in the intake of a significantly smaller blood meal in fifth instars and adults, suggesting that Rhopr-CRF/DH feeds back onto the CNS to limit gorging. When fed, mated, adult females were injected with Rhopr-CRF/DH, they produced and laid significantly fewer eggs. Finally, *in vitro* oviduct contraction assays illustrate that Rhopr-CRF/DH inhibits the amplitude of contractions of the lateral oviducts, highlighting a potential mechanism via which the hormone diminishes reproductive capacity. The study of the Rhopr-CRF/DH pathway, its components and mechanisms of action, has implications for vector control by highlighting targets to alter feeding, diuresis, and reproduction of this disease vector.

Keywords: Neuroendocrinology; Neuropeptides; Serotonin; Diuresis; Egg laying; Chagas disease

CO387

KEEPING IT ALL BALANCED: HOW LOSS OF HOMEOSTATIC REGULATION DETERMINES INSECT COLD TOLERANCE

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Cold tolerance of insects is critically important for their ability to persist in temperate and arctic environments. Little is known, however, of the proximal causes leading to cold injury in this animal group. Exposure to low temperature has been shown to coincide with depolarization of excitable tissue in many insects and it is now known that this depolarization causes loss of function (cold coma) and cellular injury in insects. The cellular depolarization observed in cold exposed insects is caused by a cold induced loss of ion and water balance. In accordance with this observation, we hypothesized that inter- and intraspecific differences in cold tolerance would be linked to the capacity of the insects osmoregulatory organs at low temperature. To test this we performed two separate sets of experiments in Drosophila and Locusts, respectively. In the first experiment we measured ion flux of Malpighian tubule and hindgut in 5 species of drosophila with markedly different cold tolerance. In accordance with our hypothesis we found that cold adapted drosophila maintain ionoregulatory capacity of at low temperature while the transport capacities of the osmoregulatory organs fail in warm adapted species. In the second experiment we used cold and warm acclimated locust system to investigate the role of acclimation on ion and water reabsorption in the insect hindgut. Here we found that cold acclimation markedly improved water reabsorption at low temperature and we demonstrate how this enables cold acclimated animals to maintain ion and water balance at low temperature. Together these two experiments demonstrate that modification of osmoregulatory capacity are highly important components of cold adaptation and cold acclimation in insects.

CO388

DISCOVERY OF A METABOLIC LINK AT THE CARBON ATOMIC LEVEL IN THE AMMONIA METABOLISM OF BLOOD-FED *AEDES AEGYPTI* MOSQUITOES

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In recent years, the successful development and application of stable [15N]-isotopically labeled compounds and tandem mass spectrometry techniques to study mosquito nitrogen metabolism have demonstrated that *Aedes aegypti* females can rapidly and efficiently detoxify high ammonia concentrations released from a blood meal digestion. Blood-fed A. aegypti females convert ammonia into Gln, Glu, Ala, Pro, and other nitrogen-containing compounds such as uric acid, allantoin, allantoic acid, and urea through the concerted action of multiple metabolic pathways. Furthermore, RNA interference studies recently uncovered that uric acid synthesis and excretion in blood-fed mosquitoes are closely regulated by cross-talk signaling mechanisms between genes that encode enzymes involved in ammonia and free radical detoxification. Those findings reveal the remarkable capacity of blood-fed mosquitoes to manage nitrogen metabolism. To verify the hypothesis that the carbon skeleton of glucose supplies the keto acids necessary for the synthesis of amino acids and other compounds during ammonia clearance in blood-fed female mosquitoes, we optimized and applied low- and high-resolution LC-MS techniques to identify [13C]-metabolites in mosquito whole body extracts. We fed mosquitoes with a blood meal supplemented with [1,2-13C2]-glucose and monitored the kinetics of incorporation of [13C]-atoms from glucose into [13C]-amino acids, [13C]-amino acid derivatives, and [13C]-organic acids during a time course (0 up to 24 h after feeding). We discovered that glucose supports ammonia detoxification through the synthesis of specific metabolites. We also found that the synthesis of these metabolites occurs through a complex interplay of multiple metabolic pathways, uncovering a metabolic link at the carbon atomic level in the ammonia metabolism of blood-fed *A. aegypti* mosquitoes.

Keywords: Mosquito metabolism, Mass spectrometry, Nitrogen waste, Ammonia, Metabolomics

CO389

HOW DO WAXWORMS EAT PLASTIC?

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Waxworms of the greater wax moth (*Galleria mellonella*) have become popularized within the scientific and public community with recent reports that they could consume plastic bags and produce ethylene glycol from the digestion of polyethylene. Curiosity into their ability to breakdown plastic bares connections to their natural diet of beeswax. *G. mellonella* are common pests to beehives, stored comb, and are commonly associated with beekeeping. It is well understood that *G. mellonella* possesses a microbiome which aids in the digestion of wax, which may also contribute to their ability to digest polyethylene. In these studies, we examine the underlying mechanisms used by *G. mellonella* to breakdown plastic as well as how feeding on plastic impacts their metabolism and fitness.

Keywords: Waxworms, Metabolism, Polyethylene

CO390

REGULATION OF THE INTAKE OF DIETARY PROTEIN AND FAT BY ADULT WORKER HONEYBEES

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Honeybees collect and eat the floral pollen of many plant species to obtain protein and fat. Pollen of many plant species is mixed together to form ‘bee bread’ within a colony, which is then consumed by adult nurse bees who feed the larvae and the queen with glandular secretions. How nurse bees regulate their intake of protein and fat simultaneously during feeding has been seldom studied. Here we use the principles of the Geometric Framework for Nutrition to study the simultaneous regulation of protein and fat in diet by newly-emerged, adult worker honeybees. We used diets composed of essential amino acids (EAA), lecithin (F), and sucrose. We measured the food consumption and survival of bees that had either been confined to eat a specific ratio of EAA-to-fat (EAA:F) or gave bees the choice of diets with different ratios of EAA:F. In both assays, we found that adult workers regulated their intake around a value of 1:2. Bees survived well on all diets, even diets containing as much as 1:12 EAA:F. Bees fed with diets high in fat were more likely to have larger hypopharyngeal glands. These data indicate that young adult worker honeybees require diets relatively high in fat compared to other bee species and other insects.

Keywords: Honeybee, *Apis mellifera*, nutrition, protein, regulation, geometric framework

Session 14.
Social Insects and Apidology

Social Immunity

CO391

FUNGUS-CONTAMINATED SOIL AND NESTING STRUCTURES IN ANT SOCIETIES

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The nest structure as well as the choice of a nesting environment will influence the exposure to pathogens as well as the risk of disease spread between its inhabitants. In this respect, ground-nesting ant societies are assumed to avoid excavating nest chambers into a substrate contaminated by entomopathogenic fungal spores that can threaten their development or even their survival. Here, we test the hypotheses that workers of the red ant *Myrmica rubra* 1) detect and avoid fungus-infected substrates, and 2) excavate nest patterns that minimize their exposure to entomopathogenic spores. We followed up during 40h the excavation of sand plates of which one half was contaminated by *Metarhizium bruneum* spores. Regardless of soil contamination, ants showed the same overall digging dynamics. However, the shape of the excavated areas markedly differed in the presence of a sanitary threat: control nests dug in a spore-free substrate showed rather isotropic patterns whereas nests faced with a fungus-infectious substrate, markedly deviated from a circular shape with a few long galleries. In contrast with our expectations, half of the colonies showed a clear-cut preference to excavate the fungus-contaminated substrate. Furthermore, these colonies were highly consistent in their choice over successive replicates. This suggests that the level of avoidance *vs* attraction for fungus spores was a striking colonial trait. Thus, our study shows that the sanitary risks associated to a digging substrate are key-factors in nest morphogenesis but do not always elicit avoidance among workers. Instead, fungal spores, at least at non-lethal doses, could be used as cues for selecting suitable nesting sites or could help ant colonies to trigger immunisation. Based on our data, we will discuss the physiological and ecological mechanisms that can explain the high consistency shown by some colonies to settle their nest in a potentially infectious substrate.

Keywords: Ants, Nest pattern, Fungus, Infected soil, Colonial identity

CO392

NF-κB SIGNALING IS IMPAIRED BY NEONICOTINOID INSECTICIDES

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Honeybee colony losses are induced by a multifactorial syndrome, associated with reduced immunocompetence and increased load of parasites and pathogens, that can be induced by different stress agents, in particular by the parasitic mite *Varroa destructor* and the Deformed Wing Virus (DWV). Recent studies have demonstrated that neonicotinoids downregulate the immune barriers of honeybees, by negatively modulating a transcription factor in the NF-κB family, which is involved in the activation of a number of defense responses. The reduced efficiency of the antiviral barriers under the Toll pathway promotes replication of DWV, which is stably associated to honeybee populations, often with asymptomatic infections. Given the central role of NF-κB in immunity, we wanted to assess the impact of neonicotinoids on both cellular and humoral components, such as encapsulation, melanization and coagulation, which may be relevant not only for pathogen control, but also for their impact on feeding activity of Varroa and its fitness. Our results show a negative impact of neonicotinoids on these immune parameters, setting the stage for studies aiming to investigate if and how their sub-lethal doses can have any influence on Varroa and other pathogens. Because NF-κB modulates a number of conserved pathways in animals, this prompted us to analyze the impact of neonicotinoids on human cell lines, in order to assess any subtle effect on non-target organisms. The transcriptional profiles obtained and the associated immunosuppressed phenotypes indicate the occurrence of a potential risk, which, however, will have to be carefully assessed at the organism level.

Keywords: Honey bee, innate immunity, pesticides, cell line

CO393

OF MITES AND BEETLES: ECOLOGICAL INTERACTIONS MODULATE SOCIAL IMMUNITY

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The costs of parental care can be alleviated or burdened by interspecific interactions on the parasitism-mutualism continuum. In the burying beetle *Nicrophorus vespilloides*, social immunity is part of parental care: parents defend their carcass breeding resource from microbes by coating it with costly antimicrobial exudates, which promote larval survival. Burying beetles also carry phoretic mites, which reproduce on the carcass alongside the beetles, sometimes with negative consequences for beetle larvae. Here we investigate the hypothesis that burying beetles may tolerate mites because they clear bacteria from carcasses and reduce the costs of social immunity, in a by-product cleaning mutualism. We found that the antimicrobial potency of beetle exudates decreases in the presence of phoretic mites, in first-time breeders, even when beetles are presented with a microbial challenge. Yet the presence of mites had no consistent effect on fitness measures such as brood size and beetle lifespan, which is not consistent with a cleaning mutualism. Using qPCR and next-generation sequencing to profile bacterial communities on carcasses, we found that mites do not reduce bacterial load on the carcasses. Instead, mites are associated with higher bacterial species richness and diversity. Our experiments suggest that mites do not reduce the costs of social immunity to beetles, but instead may alter the cues by which females decide their investment in social immunity, such as bacterial diversity.

Keywords: Parental care, lysozyme, bacterial community

CO394

USING COMPARATIVE TRANSCRIPTOMICS TO UNDERSTAND THE EVOLUTION OF IMMUNITY DURING THE TRANSITION TO SOCIALITY

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The termites are a derived eusocial lineage of otherwise non-social cockroaches. Understanding the proximal and ultimate drivers of this major evolutionary transition represents an important goal in biology. One outstanding question concerns the evolution of termite immunity, which is thought to have undergone broad-sweeping adaptations in order to enhance group-level immune protection. To understand the evolutionary origins of termite immunity, we conducted qualitative and quantitative transcriptome analyses along a gradient of sociality. Firstly, we aimed to identify large-scale genetic shifts in immune traits linked to eusociality by comparing immune gene repertoires in solitary and subsocial cockroaches and across a range of eusocial termite lineages. Secondly, we compared the responses of a solitary cockroach, a subsocial wood-roach and different castes of a lower termite species to a non-specific immune challenge, in order to understand how sociality may have influenced the evolution of immune gene regulation. Firstly, we found that termites have a broadly representative repertoire of canonical immune genes as compared to gregarious cockroaches and subsocial wood-roaches. Secondly, with respect to immune challenge, the solitary cockroach and the subsocial wood-roach displayed a similarly comprehensive induced response, while the termite response was considerably dampened by comparison and strongly influenced by caste; with reproductives displaying a generally higher constitutive level of immune-gene expression compared to sterile castes. In summary we did not find termite eusociality to be associated with significant changes in immune gene diversity, but rather to be linked with significant modifications to the regulation of immunity following the origin of division of labour.

Keywords: Termite, cockroach, caste, subsocial

CO395

GROWING UP WITH FECES: WASTE MEDIATED STARVATION RESISTANCE & SOCIAL IMMUNITY IN AN INSECT WITH FACULTATIVE MATERNAL CARE

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To combat increased infection risk, group living animals can mount collective defenses known as social immunity, which include the spread of antimicrobial exudates and sanitary behaviors such as waste management. Well studied in obligatory and permanent forms of sociality, the occurrence and role of these mechanisms in less derived, facultative social systems – and thus, in the early evolution of sociality – remains largely unclear. Here, we shed light on the curious absence of sanitary behavior in the European earwig, an insect with temporary family life and facultative maternal care, and how the accumulation of feces in the nest provides unique benefits to earwig offspring (nymphs) in terms of starvation resistance and pathogen defense. In several experiments, we tested 1) whether access to maternal or nymph feces affects nymph survival under food deprivation. We then 2) examined whether nymphs prefer food to feces, and if they discriminate between feces from mothers and nymphs, related and unrelated. Finally, we 3) conducted inhibition zone assays to investigate anti-microbial properties of the feces against two fungal and three bacterial species, and whether any such activity depends on feces producer and/or previous family interactions. We found that when deprived of food, access to sibling feces, but not mother feces, enhanced offspring survival. When choosing between food and feces, nymphs preferred regular food to feces, but nevertheless always consumed some feces. They did not discriminate between feces produced by nymphs or mothers, related or otherwise. Intriguingly, we finally found that that earwig feces inhibit the growth of two bacteria and two fungi, and that that family interactions inhibited the antibacterial activity of maternal feces against one bacteria. Overall, we show that maintaining feces within the nest not only promotes the maintenance of family life with nutrients, but also improve pathogen defense as a form of social immunity.

Keywords: Social immunity, Family life, Feces, Precocial, Earwig

CO396

SCAVENGING BEHAVIOUR BY ANTS DEPENDS ON THE PREY INFECTIOUSNESS

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The limitation of pathogens' entry inside the nest is a first line of sanitary defence displayed by social insects. In scavenging ant species, one may expect that foragers should be able to detect and discriminate preys that are possible sources of infection. Here we challenged *Myrmica rubra* workers with choice tests between two preys, one of which being infected with the entomopathogen fungus, *Metarhizium brunneum*. In addition to control preys, we offered dead fruit flies (1) that were covered with a controlled concentration of conidia (5.0×10^6 spores/mL), (2) that recently died from *M. brunneum* infection or (3) of which the dead body was covered by sporulating *M. brunneum* fungus. The presence of spores did not reduce the retrieval rate of prey by ants nor the time they spent antennating it. Likewise, the ants do not seem to early detect possibly infectious prey as they equally retrieve preys regardless of whether they died from a fungus infection or not. However, we found that ants refrain from taking sporulating prey that was significantly less chosen than control prey. Furthermore, ants spent twice less time exploring the sporulating body than the control, most probably to limit their exposure to this sanitary threat.
Our results show that foraging ants are actually able to tune their scavenging activity according to the sanitary risks associated to preys. This sanitary barrier appears to be very efficient against sporulating insects which represent an instantaneous highly infectious material. By contrast, the absence of early discrimination of spore-bearing prey or of prey died of illness suggests that other physiological defences may be sufficient to protect the ants against disease outbreak.

Keywords: Sanitary strategies, ants, pathogen, prey, choice test

CO397

SUPERORGANISM IMMUNITY: HOW ANTS BEHAVE LIKE IMMUNE CELLS TO ERADICATE INFECTIONS

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Social insects colonies are often thought of as single organisms due to the division of labour within their colonies, where the majority of individuals forgo reproduction to help one or a few fertile kin to produce offspring instead. When this separation of sterile and reproductive individuals becomes irreversible - as is the case for some termites, bees, wasps and ant lineages - it mirrors the separation of soma and germ line cells in a multicellular organism and the colony can be called a "superorganism". In this talk I will discuss how this transition necessitated the evolution of an immune system-equivalent to protect superorganisms from disease, which is present in social insects colonies as collectively performed disease defences, known as social immunity. Specifically, I will present our recent work on an ant host-fungal pathogen system, where we uncover how invasive garden ants (Lasius neglectus) detect and eradicate infections (Metarhizium) before they can establish and transmit within the colony. At both a mechanistic and functional level, this behaviour has many striking analogies to the elimination of infected cells by the immune system of multicellular organisms, and has likely evolved for the same reasons: ensuring the survival of the whole (the body/colony) over its disposable parts (single cells/workers). Our data therefore suggest that common evolutionary processes were at play during the emergence of both multicellular organisms and superorganismal insect colonies.

Keywords: Social insects, disease, colony, infection, social immunity, superorganism

CO398

DWV-CARRYING VARROA MITES AFFECT THE HONEYBEE CORE BACTERIAL COMMUNITY

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Varroa destructor host-feeding behaviour and the consequent virus transmission are major concerns in infested bee hives. In order to counteract the action of this parasite and of the pathogenic viruses it carries, honey bees can count on a variety of defence mechanisms including their innate immune response, a specific hygienic behaviour, and a still not well understood contribution of their core microbial community. The latter is mostly represented by bacteria belonging to the following taxa: *Gammaproteobacteria*, *Betaproteobacteria*, *Lactobacillus spp.*, and *Bifidobacterium spp.*, that reside in the gut. In the present study we compared the immune-related gene expression level and the resident bacterial community abundance in different bee stages from infested *versus* non-infested colonies. As expected, infested colonies were characterized by a higher deformed wing virus (DWV) titer and a generally reduced level of immune gene expression. Significant quantitative changes were also observed on the core bacterial community. Regardless of the colony of origin, emerging honeybees bearing a sucking mite on their body showed a significantly higher DWV titer and a greater immune-suppression compared with insects without mites. A significant increase in the target bacterial taxa was detected on these adults, thus suggesting a possible role of the microbial community to compensate a compromised state of health.

Keywords: Mite, DVW, AMP, Immunosuppression, *Lactobacillus*, *Bifidobacterium*, Snodgrassella, Social immunity.

CO399

SOCIAL IMMUNITY: REGULATION OF COLLECTIVE DISEASE DEFENCES

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Social insects have evolved collective disease defences (social immunity) in response to an exacerbated risk of disease outbreaks associated with their social lifestyle. Recent years have witnessed a steady increase in studies revealing examples of social immunity from a growing number of study species. While this enables comparative studies of defences across different species, it also highlights areas of this young research field that we know relatively little about. For example, it is well recognised that behavioural adaptations are an important component of social immunity, including prophylactic and on-demand sanitary care of nestmates and brood, and moribund individuals leaving the nest to die in isolation. Yet, our knowledge of how these measures are regulated and modulated is limited; *e.g.* are inducible defences mainly triggered by cues from the pathogen or from the infected host? I will discuss how studies combining behavioural, molecular and chemical approaches can help to advance our understanding of how collective disease defences are regulated.

Bees: Management, Ecosystem Services, and Products

CO400

INVESTIGATING THE IMPACT OF AGRI-ENVIRONMENT MANAGEMENT INTERVENTIONS ON DISEASE TRANSMISSION IN BUMBLE BEES

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Wildflower strips are an important component of agri-environment schemes, used as a management tool to enhance wild bee populations by providing forage on agricultural land. These interventions have been shown to increase wild bee abundance. However, recent work also suggests that flowers may act as a hub for the transmission of viruses and other parasites between bees, both within and across species. As wildflower strips are often islands of forage within an otherwise relatively barren agricultural landscape, these interventions are likely to concentrate pollinators in these areas. This increase in density will reduce the time between flower visits and therefore has implications for the rate of parasite transmission, as it may reduce the amount of time parasites are exposed to environmental degradation. Given the role that parasites are thought to play in bee population declines, we asked how bee density affects transmission of two common bumble bee parasites, *Crithidia bombi* and slow bee paralysis virus (SBPV). Here we present the results of a large-scale semi-field experiment, carried out in 2017 at NIAB EMR in Kent, UK. Using polytunnels containing native wildflowers in 7x8m compartments, we compared the impact of low and high bee density on transmission. The spread of *C. bombi* and SBPV from

an infected donor colony to uninfected recipient colonies was monitored over the course of 4 weeks. We will discuss the evidence that bee density and drifting between colonies influence the transmission of parasites, and the implications of this for the value of wildflower strips on farmland.

Keywords: Agri-environment scheme, wildflower strip, disease transmission, parasite transmission, bee health

CO401

AMINERGIC UNDERPINNINGS OF PHEROMONAL MODULATION OF OLFACTORY LEARNING AND MEMORY FORMATION IN HONEYBEES

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Pheromones are chemical messengers allowing the exchange of information between members of the same species. Besides releasing stereotyped behavioural and/or physiological responses, recent reports show that pheromones modulate responsiveness to stimuli commonly used as reward and punishment in learning protocols, thus suggesting that they could modulate learning and memory formation. Here we explored this possibility in the honeybee *Apis mellifera*. We studied the effect of one pheromone signalling valuable resources (geraniol) and two alarm pheromones signalling noxious situations (isopentyl-acetate (IPA) and 2-heptanone (2H)) on an olfactory differential conditioning in which one odour was paired with sucrose and another odour remained unrewarded. Bees were exposed to the pheromones either before or after conditioning to evaluate their impact on acquisition and memory retention, respectively. We found that bees exposed to geraniol before conditioning not only improved their learning performance, but had also better memory retention than controls up to 24h. By contrast, 2H strongly inhibited appetitive learning and memory retention up to 72h following conditioning with respect to controls. IPA did neither modulate learning nor memory performance. None of the three pheromones affected memory retention evaluated at 2h, 24h and 72h in bees exposed after conditioning, suggesting that pheromones affect memory formation but not its retrieval. To explore the underlying neural mechanisms of this modulation we used a pharmacological approach to specifically block/activate the octopaminergic and the dopaminergic circuits, which signal appetitive and aversive events in the bee brain respectively. Injections of octopamine (OA), dopamine (DA) and their antagonists were performed into the brain of bees that were exposed to pheromones before conditioning. As OA and DA-antagonist restored learning and memory in 2H-exposed bees, and DA- and OA-antagonists counterbalanced the geraniol effect on learning and memory, we concluded that both aminergic systems underpin the pheromonal modulation of learning and memory in honeybees.

Keywords: *Apis mellifera*, Biogenic amines, Behavioural plasticity, Insect cognition, Memory, Olfactory Learning, Pheromones

CO402

SOCIAL INFORMATION USE IN CAVITY NESTING BEES

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Animals often face unpredictable situations where the decisions has to be made on limited information. To optimize decisions, individuals constantly need to update their information about the environment, either by sampling it by themselves using a trial-and-error strategy, or by observing and relying on the behaviour, presence or performance of other individuals, generally termed social information use. Recent studies suggest that individuals use also heterospecifics as information sources if they are ecologically similar and the cost of competition does not outweigh the benefits of information use. Despite this strategy is well documented in social species from insects to apes, little is known about social information use in non-social animals. Here, we tested with a field experiment whether solitary bees from the family Megachilidae use earlier nesting bees (the red mason bee *Osmia bicornis*) as information sources in their nest-site decisions. The information was provided by using artificial nests that mimic the natural nests of earlier nesting bees. Artificial nests were built by using wooden blocks with a drilled hole covered by mud. The success of earlier nests were manipulated by presenting an entrance fully covered by mud (successful) or mud coverage with small holes drilled through it (mimicking parasitized nest). Symbol (circle or triangle) on a black background was painted around the entrance, while the alternative symbol was painted on an adjacent empty nest. This design creates an apparent symbol choice of the earlier nesting bee. Two equivalent empty nests adjacent to each other and differing by the type of symbol, were positioned at a distance from the referred nest. We found that bees used social information selectively and copied and rejected symbol choices of successful and parasitized earlier nesting bees, respectively. Our conclusion is that selective interspecific social information use may lead to dynamic co-existence of competing species.

Keywords: Social information use, solitary bees, nesting site, parasitism

CO403

DECODING THE IMPACTS OF LAND USE PATTERNS ON BEE HEALTH

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Bees are critical pollinators of agricultural crops, but populations of both managed and wild pollinators are in decline globally. Multiple interacting factors are driving pollinator declines, including parasites and pathogens, as well as biotic and abiotic features of the landscape, such as the availability of forage, pesticide use, population densities, and climate. I will summarize our studies using genomic approaches, behavioral studies, and spatial ecology to understand how these factors interact to influence bee health and to build predictive models of bee health that beekeepers, land managers, growers, and policymakers can use to evaluate the quality of their landscapes for supporting bee populations and obtain recommendations for improving their landscapes and management practices.

Keywords: Nutrition, pesticides, pathogens, health

CO404

CONSTRUTION AND RESCUE OF INFECTION CLONE OF ISRAELI ACUTE PARALYSIS VIRUS

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Honeybee is fundamental to supply the pollination servicefor increasing the agricultural production and biodiversity. Recently in America and European countries, however, honeybee colony went through a large number of losses that has been linked with a RNA virus, Israeli acute paralysis virus (IAPV).Current knowledge about honey bee virus is limited, especially on virulence and pathogenicity of IAPV due to the lack of honey bee virus cell. Thus, it is crucial to construct a reverse genetic system to understand clearly the infection and develop effective drug to control IAPV. For this purpose, we constructed a full-length genomic cDNA clone of IAPV and identified the on healthy adult worker bees. To further study the effect of natural product on inhibition of IAPV replication, we injected the healthy adult bees with constructed infectious IAPVand investigated the effect of Q application on their survival.Our results indicated that we not only constructed infectious clone of IAPV with virulence but also found one agent based on natural product to control the IAPV infection. Thus, we provided a power tool to study the molecular mechanisms involved in viral genome replication and virus pathogenesis, and found a potent antiviral agent that can be used widely in field. These results pave the way for further study the infection mechanism of honey bee virus as well as for antiviral treatment of bee viruses infected hives in practice. To our knowledge, our study provides the first infectious clone and antiviral agent based on the natural product and established a general model platform for studying the genetic characterization and gene functions of honey bee viruses.

Keywords: Isralei acute paralysis virus, antiviral activity, infectious clone, natural product

CO405

IPM TACTICS IN *VARROA DESTRUCTOR* CONTROL IN HONEY BEES: CHALLENGES AND SUSTAINABLE SOLUTIONS AS A RESULT OF ENVIRONMENTAL CHANGES

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Current beekeeping challenges call for beekeepers to have a flexible management approach. Many data show that environmental changes have a direct influence on honey bee development. Climate influences plant physiology, including nectar and pollen production, which are directly linked with honey bee colonies' foraging activity and health. Besides that, the ectoparasitic mite *Varroa destructor* (Anderson & Treuman) seems to be able to easily adapt to any situation: its, global spread has resulted in the death of millions of honey bee (*Apis mellifera*) colonies during the past 50 years. On a practical perspective, during the last decade substantial progress has been made and a wide range of different chemical and organic active ingredients, formulations and application techniques to reduce mite populations have been registered and made available to beekeepers. Here we review present alternatives available, including two new bio-mechanical management techniques to be applied during early spring (late February) that were tested with the goal to assess their impact on colony development, mite population growth and honey production during six months following the treatments. Our results indicate that queen caging for twenty-one days at the beginning of the season, significantly affect mite reproduction resulting in a lower final varroa mite infestation level, withouth affecting honey harvest, thus adding a new weapon to honey bee management strategies. Our results emphasize the need to differentiate the interventions based on seasonality due to unpredictability seasonal climatic trend. We also recommend to consider this method as an alternative in the annual varroa control strategy, to avoid surprises during the harvest season due to ineffective treatments of the previous year or robbing and drift episodes in the fall.

Keywords: Honeybee, *Varroa destructor*, disease, environmental changes, control

CO406

HAEMOLYMPH REMOVAL BY THE PARASITE *VARROA DESTRUCTOR* CAN TRIGGER THE PROLIFERATION OF THE DEFORMED WING VIRUS IN MITE INFESTED BEES (*APIS MELLIFERA*), CONTRIBUTING TO ENHANCED PATHOGEN VIRULENCE

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The association *Varroa destructor* - Deformed Wing Virus is regarded as a major cause of worldwide honey bee colony losses. The mite acts as a vector of the viral pathogen and can trigger its replication in infected bees; however, the causes of viral proliferation in mite infested bees are still poorly understood. Here we develop and test the hypothesis that mite feeding destabilizes viral immune control through the removal of both viral particles and immune effectors. Consistent with this hypothesis, we show that the experimental removal of increasing volumes of haemolymph from individual bees results in increasing viral densities. This novel hypothesis, grounded in ecological predator-prey theory, provides the most suitable explanation for the data we collected under controlled lab conditions, whereas, we found little support for alternative proposed mechanisms of viral expansion via mite immune-suppression or within-host viral evolution. This new model of the pathogen-parasite interactions in bees deepens our understanding of the mechanisms underpinning honey bee health decline and colony losses.

Keywords: *Varroa destructor*, Deformed wing virus, *Apis mellifera*, Volterra model, pathogen virulence, viral replication

CO407

DEFORMED WING VIRUS OF THE HONEY BEE: A MORE VIRULENT GENOTYPE IS TAKING OVER EUROPE

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It is widely, if not universally, accepted that the major threat facing honey bees (*Apis mellifera*) in northern temperate climates, including Europe, is the ectoparasitic mite *Varroa destructor* (varroa) and the viruses it transmits between honey bees. Chief amongst these viruses is Deformed wing virus (DWV), which comprises a complex of at least two predominant genotypes (named A and B), and which is likely the cause of elevated overwinter

mortality since the arrival of *V. destructor* to Europe within the last 50 years. There is also considerable sharing among co-occurring bee species of DWV, suggesting it may negatively impact populations of wild bee species. Using my own group's and colleagues original data, I will provide evidence for four points; that (i) DWV is the most widespread and prevalent virus associated with honey bees infested by varroa mites; (ii) DWV is causal in honey bee overwinter mortality; (iii) DWV genotype B is more virulent that the original DWV genotype A; and (iv) DWV genotype B is spreading in distribution and prevalence. As it does so, individual hosts may become co-infected by DWV genotypes A and B, opening up the possibility for viral recombination. I will explore the extent to which recombination among DWV genotypes also plays a role in the pathology of DWV and its evolutionary trajectory, with consequences for apiculture and the conservation of wild bee species.

Keywords: Honey bee, virus, wild bee species, virulence, recombination

CO408

INVESTIGATING THE INFLUENCE OF FLORAL RESOURCE AVAILABILITY ON THE NESTING BEHAVIOUR OF THE SOLITARY BEE; *OSMIA BICORNIS*

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Many factors have been implicated in affecting pollinator decline. The contribution of nutritional effects of floral resources at both the individual or population level, however, requires further study. The UK National Pollinator Strategy aims to boost the number of pollinating insects on commercial farms by creating specific habitats tailored to local conditions and native insects. There is currently, however, limited understanding of the suitability of floral resources for solitary bees, an important group including many early pollinating species. This study investigates selective floral utilisation by *Osmia bicornis*, when foraging in either floristically enriched or unenriched habitats established as part of government funded stewardship schemes. Within each habitat *O. bicornis* were released and allowed to forage freely. Weekly vegetation surveys were conducted and rate of larval cell construction by foraging bees assessed. Pollens present in larval cell provisions were identified to species, quantified and compared with relative availability of different flower species during the period of cell construction. Amino acid analysis was undertaken, establishing the quality of pollen mixes in larval provisions. Laboratory studies were conducted to support interpretation of field data by comparing development success and overwintering survival of larvae fed on pollen mixes taken from larval cells constructed by wild bees, or artificial pollen mixes constructed to investigate specific hypotheses. The composition of plant species within wild nest cell pollen provisions was not directly correlated with the most common flowers, and shows a bias towards specific species. This provides a basis for understanding the observed high levels of selectivity and the identification of potential "keystone" species. The "keystone" plant species highlighted in this study are not specifically addressed in current conventional agri-environment schemes. Thus, in order to support more diverse pollinator assemblages, future schemes could be modified to include these wild plants, along with enhanced hedgerows and woodland environments.

Keywords: Pollinators, *Osmia bicornis*, Solitary bee, Nutrition

Symbiosis in Social Insects - Behavioural and Ecological Interactions

CO409

UNVEILING COMMUNITY PATTERNS AND SPECIES TROPHIC NICHES OF TROPICAL AND TEMPERATE ANTS USING AN INTEGRATIVE FRAMEWORK OF FIELD DATA AND TROPHIC MARKERS

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How species use and share food resources is a key aspect of community functioning. However, there is little information available on trophic niche for most species, particularly in tropical communities. Moreover, few studies integrate the source-specific field observations with the temporal scope of indirect techniques. In this work, we assessed resource use and partition among ant species in a Brazilian Atlantic forest and a German temperate forest. Trophic niches were assessed with: baits, using a protocol designed to assess use of multiple resources (23 species in Brazil); trophic position inferred from stable isotopes of nitrogen and carbon (31 species); fatty acid (NLFAs) composition, used here for the first time to study ants in the field (41 species). In Germany, seven species were assessed with all methods. We analyzed data with network analysis, multivariate and Mantel tests. Network indexes for baits (specialization – H2'; modularity) were similarly low for both communities, suggesting high level of generalization independent of species number. NLFA compositions differed between communities, being more generalized in Germany, where C18:1n9 was largely predominant. In Brazil, bait use and NLFA composition were related, as well as species' specialization indexes (d2') for both methods. Trophic position was also correlated to bait use and NLFA composition. In Germany, no correlations were observed, likely due to the small species number. Our results showed for the first time a direct relationship between field observations and NLFAs stored in ants' fat body, and that both are related to long-term trophic position of the ants. Nevertheless, species with distinctive behaviors, such as *Wasmannia auropunctata* and *Lasius fuliginosus*, could be understood only by complimentary results provided by different methods. An integrated framework of stable isotopes, NLFAs and field observations combines source specificity and temporal representativeness, providing valuable information to understand communities and species niches.

Keywords: Formicidae, Atlantic forest, temperate forest, fatty acids, isotope analysis, baits

CO410

THE INTERSPECIFIC NESTING RELATIONSHIP OF *PARAPONERA CLAVATA* AND *PARTAMONA TESTACEA*

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Social insects are commonly involved in interspecific nesting associations. The association with aggressive species can provide relatively harmless species with an effective shield against predators, especially in the extremely diverse environment of tropical forest. Hosted species should rely on fine mechanisms to elude the host colony defense and, eventually, be involved in a reciprocal exchange of benefits. These intimate relationships represent profitable models in behavioral ecology mostly when the two parties are represented by eusocial species. This study conducted in the Urku center (San Martin, Peru) describes the first association of the giant tropical ant *Paraponera clavata* with a social bee (the stingless bee *Partamona testacea*). This stingless bee is reported to occasionally nest in association with termites and ants of the genus Atta. *Paraponera clavata* is a generalist predator of many invertebrates with a notorious ability in patrolling and nest defense. The acceptance by these ants of a stingless bee nesting in its own home raises many questions about the possible mechanisms triggering and maintaining these associations. To describe the interaction between *P. clavata* an *P. testacea* we integrated ethological and biochemistry data. Behavioral experiments showed that ants are less aggressive against bees belonging to the hosted colony. Guard bees at the nest entrance avoid ants but, when they try to enter the hive, bees fatally react against the intruders regardless of the colony they belong to. To unveil potential proximate factors determining intercolonial recognition, the similarity of cuticular hydrocarbons profiles characterizing bee and related ant host colonies have been verified by gas-chromatography/mass spectrometry. This allowed to understand the relationships between colonies and discern the proximate and ultimate causes determining the associations of these social insects.

Keywords: Nesting association, chemical recognition, bullet ants, Meliponini

CO411

ENHANCEMENT OF CHRONIC BEE PARALYSIS VIRUS LEVELS IN HONEYBEES ACUTE EXPOSED TO IMIDACLOPRID: A CHINESE CASE STUDY

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Though honeybee populations have not yet been reported to be largely lost in China, many stressors that affect the health of honeybees have been confirmed. Honeybees inevitably come into contact with environmental stressors that are not intended to target honeybees, such as pesticides. Although large-scale losses of honeybee colonies are thought to be associated with viruses, these viruses usually lead to covert infections and to not cause acute damage if the bees do not encounter outside stressors. To reveal the potential relationship between acute pesticides and viruses, we applied different doses of imidacloprid to adult bees that were primarily infected with low levels (4.3×10^5 genome copies) of chronic bee paralysis virus (CBPV) to observe whether the acute oral toxicity of imidacloprid was able to elevate the level of CBPV. Here, we found that the titer of CBPV was significantly elevated in adult bees after 96 h of acute treatment with imidacloprid at 66.9 ng/bee compared with other treatments and controls. Our study provides clear evidence that exposure to acute high doses of imidacloprid in honeybees persistently infected by CBPV can exert a remarkably negative effect on honeybee survival. These results imply that acute environmental stressors might be one of the major accelerators causing rapid viral replication, which may progress to cause mass proliferation and dissemination and lead to colony decline. The present study will be useful for better understanding the harm caused by this pesticide, especially regarding how honeybee tolerance to the viral infection might be altered by acute pesticide exposure.

Keywords: Imidacloprid, Chronic bee paralysis virus, Synergistic interaction, *Apis mellifera*, Environmental stressor

CO412

BEHAVIOURAL AND ECOLOGICAL FACTORS SHAPING THE GUT MICROBIOME IN ANTS

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Social insects entertain symbiotic interactions with many different groups of organisms, such as plants, fungi and bacteria. Endosymbionts and the gut microbiome have received more attention in recent years with the advent of new techniques. The gut microbiome has been shown to play an important role in nutrient provisioning but also in social insect health. For a number of social insect taxa core microbiota carried by most individuals of a species have been identified, suggesting that mechanisms have evolved to maintain and regulate the gut microbiome. While identification of bacterial lineages carried by social insects has made great progress the understanding of the factors shaping the gut microbiome is lacking behind. I will present an overview of our current understanding of behavioural and ecological factors shaping the gut microbiome with a focus on ants.

Keywords: Gut microbiome, crop, Camponotus

CO413

WHAT FLORAL RESOURCES HONEYBEES (*APIS MELLIFERA*) USE IN URBAN HABITATS? THE CASE OF GENEVA, SWITZERLAND

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Use of pesticides, habitat fragmentation and landscape simplification tend to endanger pollinator’s survival in agricultural habitats. Urban habitats are thus seen as potential refuges for pollinator populations because they present favourable environmental conditions. However, few data are available on the specific resources that bees use in urban areas. In this study, we looked whether plant diversity used by pollinators is different between urban and agricultural habitat, taking domestic bee (*Apis mellifera*) as a model. We hypothesized that: 1) resources used in both habitats are different and 2) urban habitat includes larger plant diversity. We sampled pollen loads in 15 hives in urban and agricultural habitats, between spring and autumn 2016 using pollen traps. Traps were collecting pollens during 24 hours every two weeks. Pollen loads were then weighted, counted and identified to the furthest taxonomical levels using morphological criteria under a microscope. Preliminary results indicate that quantity and diversity of the pollen sampled is not significantly different between agricultural and urban habitats. Nevertheless, bees seem to use different species in both habitats probably because different plant communities are present. Moreover, we observed that ligneous vegetation is an important resource in terms of pollen in both habitats. These results show that urban zones can be interesting for pollinator populations because they display diverse and abundant

plant communities. However, more research is needed to better understand the relationship between domestic bees and all other pollinator species. Policies of green spaces management caring for healthy tree communities comprising resource-rich species is also crucial for bee's survival. Promoting semi-natural habitats such as wild flowers meadows in urban green spaces could significantly help pollinator populations to survive and develop. In this sense, the BEEFLORA project will test in 2018-2019 the attractiveness of a melliferous native flower blend on all bee species and pollinators.

Keywords: Pollinator, urban habitat, bees, plant diversity, pollen resources, beekeeping

CO414

REBEL WORKERS IN HONEYBEE HAVE TENDENCY TO BECOME INTRASPECIFIC REPRODUCTIVE PARASITE

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The honey bee is one of those species in which intraspecific reproductive parasitism can exist and workers are sometimes found reproducing in their own or foreign colonies. In this research, we compare tendency to be intraspecific parasite between normal honey bee workers characterized by not so high reproductive potential, and recently discovered new subcaste of honey bee workers, called 'rebel workers', characterized by high reproductive potential. We expected that this high reproductive potential of rebel workers can influences their life strategy and these individuals can more often drift to other colonies and lay their own eggs. The result confirm our expectation and showed that 20.5-38.9% rebel workers drifted to other foreign colonies while only 2.6-7.6% normal workers did the same (P=0.0002). The rebel workers also preferred to drift to queenless colonies than queenright (P=0.0304) while the normal workers didn't show this preferences (P=0.8845). The rebels had also more ovarioles in the ovary than normal workers (P<0.001) and their ovarioles was more often activated than in normal workers (P<0.001). The number of ovarioles and their activation in normal workers were not differ between individuals collected from different type of colonies (P>0.05), while between the rebel workers these parameters were statistically significant and depends on type of colonies were workers were caught (P<0.001). Rebels with more ovarioles in ovary preferred drift to queenlees colony, rebels with less ovarioles drifted to queenright colony while those with the least number of ovarioles stayed in own colony. These results showed that rebels have tendency to reproductive parasitic behavior which also could enhance to maintenance of rebel workers strategy in bee population.

Keywords: Honeybee, social insect, intraspecific parasite, reproduction, rebel workers, life strategy

CO415

HOST COMMUNICATION SIGNALS DIVERSIFY AS THE RESULT OF COEVOLUTIONARY INTERACTIONS WITH SOCIAL PARASITES

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Understanding the evolution of communication signals is a central topic in evolutionary biology, and co-evolutionary interactions between social parasites and their hosts can result in signal variation between geographically separated populations. In social wasps of the genus *Polistes*, facial marks are visual signals of fighting quality (*i.e.*, badge of status). However, experimental tests have given conflicting results, suggesting geographical variation in the use of facial marks between and within species. In a long-term field study on *Polistes biglumis*, I tested 1) whether facial marks were used as signals in competitive contests, such as when foundresses defended their nests against conspecific nest-usurpers; 2) whether these signals were consistent across 3 geographically separated populations; and 3) whether signal variation among populations was associated with the prevalence of obligate social parasites (*Polistes atrimandibularis*). There was a large individual variation in facial marks both within and between populations. Conspecific nest-usurpers had consistently more yellow in their face than the foundresses they targeted, suggesting that facial marks conveyed information about fighting quality and are used as badges of status. However, this result held exclusively in the social-parasite-free population. In contrast, usurpers and foundresses had random facial marks in the other two populations, which were infested by obligate social parasites, suggesting that facial marks were not functioning as signals there. Facial traits of obligate social parasites might have interfered in host visual communication, contributing to diversifying the use of facial marks between populations and suggesting a role for social parasites as agents of selection on their hosts.

Keywords: Social wasps, Polistes, visual signals, facultative parasites

CO416

COMPARATIVE NETWORK ANALYSES OF OBLIGATE INTERACTIONS BETWEEN ANT ASSOCIATES AND THEIR HOST ANTS IN EUROPE

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Ants are key partners in an extremely large number of symbiotic relationships with ant associates. Interestingly, these relationships are highly variable in terms of host-specificity and span the entire mutualism-antagonism continuum. A number of local field studies examined network characteristics and dynamics of ant-ant associates at a local scale, but a large scale analysis integrating and comparing different types of ant associations is currently lacking. Therefore, we did an exhaustive literature study on all known ant associations of obligate myrmecophiles throughout Europe, comprising five types of ant associates: aphids, nematodes, fungi, myrmecophiles and social parasites. This study resulted in a binary interaction matrix with about 700 obligate ant associates, 170 ant species and 2500 interactions between them. Ant genera were highly different with regards to the number of associates, the proportional distribution of interactions with different types of ant associates and the number of unique associates. The number of interactions per host ant species, corrected for sampling effort, was positively correlated with its colony size and nest characteristics. The interactions were nested for all types of ant-associates. We found that phylogenetic-related hosts interacted with similar communities of associates. In case of multiple hosts, we found that a social parasite targeted closely related hosts, while an aphid and a myrmecophile interacted with a much broader taxonomic distribution of hosts. Overall, we demonstrate that integrating different types of symbionts in one ecological network study could reveal contrasting patterns in network structure, host specificity, host distribution and phylogenetic signals.

CO417

DISEASE-FREE MONOCULTURE FARMING BY FUNGUS-GROWING TERMITES

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The ancient Old World fungus-farming termites have a sophisticated agricultural symbiosis that pre-dates human farming by 30 million years, produces no organic waste, and, in stark contrast to most other organisms, does not suffer from specialised diseases. In this talk, I will summarize our current understanding of how this is accomplished through integrated farming practices involving behavioural specialisation in the termite host, complementary contributions from the fungus crop and co-diversified bacterial communities to near-complete plant substrate decomposition, and the presence of a series of consecutive and complementary defences that collectively appear to prevent the introduction and successful establishment of disease.

Keywords: Macrotermitinae, Macrotermes, Odontotermes, Termitomyces, (meta)genomics, metabolomics

CO418

EMERGING BEE VIRUSES: FROM MOLECULES TO HOST AND VECTOR ECOLOGY

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Technical advances in the study of molecular evolution have crystallized the fundamental insight that many bee pathogens evolve and adapt over timescales that overlap with host ecology. At the same time, the role played by bee host community ecology is increasingly being appreciated in host-parasite interactions. Here, we focus on Deformed wing virus (DWV) and present recent studies exploring the link between virulence, DWV genetic diversity and changes to host ecology - namely the arrival of the invasive vector, the *Varroa destructor* mite that vectors viruses in the honeybee, *Apis mellifera*. Specifically, we show how *V. destructor* may have created conditions for the emergence of more virulent strains of DWV. We present a molecule-to-ecology framework to help interpret findings and to guide hypotheses, emphasizing the role of molecular interactions between viruses and host immunity as drivers of change at the bee population level.

Session 15.
Urban Entomology and Stored Product Protection

Integrated Pest Management in Stored Product Protection

CO419

PHYSICAL METHODS IN MODERN STORED PRODUCT IPM

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In the last two decades the number of available pesticides for stored product protection decreased and a number of compounds (*e.g.* MeBr, Dichlorvos, Pirimiphos-methyl) are no longer available due to environmental or health concerns. Moreover, the consumer does not tolerate residues in food and organic agriculture has gained importance starting out from the Nordic countries. This leaves farmers with little choice but to invest in preventive methods. Pest-proof warehouses and silo-bins are such a method but require dry grain in order to avoid condensation and mould. Grain drying and early grain cooling are further physical and preventive methods. Hermetic enclosure in silobags, bigbags or vacuum storage are additional options if there are no proper solid structures available. The grain drying involves heat, another option to disinfest dry goods or empty structures. Cooling will interrupt insect development below temperatures of some 13°C but control of all stages is possible only if temperatures are below minus 18°C. Pest detection in stored products is possible by physical means such as temperature monitoring or acoustic surveillance. The latter may be combined with biological control in bulk storage and thus make bio-control much more effective. Physical pest control is possible with extreme temperatures (>45°C or below -18°C) with radiation or impact mills (entoleters). A new project will analyse optic surveillance in combination with the use of laser beams to control insects in premises of food industry. New technologies require research and many European countries carelessly neglected stored product protection and other applied research. This needs to change if we want to secure nutrition in a world-wide scale.

Keywords: Pest-proof, hermetic, vacuum, drying, cooling, heat, optic surveillance, acoustic detection, impact, laser

CO420

USING WIRELESS PHOSPHINE SENSORS FOR STORED PRODUCT INSECT CONTROL IN LARGE-SCALE INDUSTRIAL FUMIGATIONS

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We evaluated wireless sensors in order to illustrate gas concentration, but also to depict spatio-temporal distribution of the gas within a given facility and commodity. Moreover, this novel technique is able to evaluate the commodity penetration speed in "real world" fumigations, its distribution attributes, the effects of temperature and relative humidity and the effectiveness of the dosages used in commercial applications. Overall, we carried out tests in more than 100 "real-world" fumigations, in facilities ranging from ships to flourmills and containers and commodities ranging from grains and flour to dried fruit and tobacco. Based on our results, distribution in large warehouses is not usually adequate for a satisfactory level of insect control and any application will not lead automatically to uniform concentrations unless means of aeration/circulation are used. In fact, in light of our findings, it is likely that, in some of the cases tested, gas penetration in the tested commodities is minimal, suggesting that phosphine may not be effective. One of the critical factors is that some biotic and abiotic conditions may have different weight per fumigation, *i.e.* often temperature may have less important role in phosphine concentration and distribution of phosphine than humidity or the commodity that is to be fumigated. As a result, concentrations are either over- or underestimated, with the concomitant implications in the expected insect control. Finally we found that increased concentrations cannot be used in some cases due to limitations of the free air and free humidity in a closed space. Overall, these tests that were carried out for the first time globally with phosphine sensors clearly suggested that the sensors were effective in measuring phosphine and are generally expected to play an important role in the near future in programs related with integrated pests management at the post-harvest stages of agricultural commodities.

Keywords: Phosphine fumigation, monitoring, wireless sensors, stored product insect control

CO421

THE BIOACTIVITY OF *CLINOPODIUM SPP.* ESSENTIAL OILS ON *SITOPHILUS ZEAMAIS*: A MORPHOLOGICAL, BEHAVIORAL AND ELECTROPHYSIOLOGICAL STUDY

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Among foodstuff pests, *Sitophilus zeamais* (Motschulsky) is considered as one of the most destructive. The use of plant-derived essential oils (EOs) is considered as a promising tool for pest control, due to their positive properties in terms of efficiency, low toxicity for mammalians and low environmental impact. In particular, OEs extracted from Lamiaceae are considered among the most bioactive in terms of repellent and/or insecticidal effect. We investigated the repellence of the essential oils extracted from two aromatic plant species from Ecuador's flora, belonging to the genus *Clinopodium*, *i.e. C. tomentosum* and *C. nubigeum*, towards adults of *S. zeamais*. Behavioral assays carried out at different concentrations ranging from 0.6 to 10% in 2-way static olfactometer showed a repellent effect of the two EOs with results statistically significant at the higher concentrations of 5% ($P=0.001$) and 10% ($P<0.001$). We also carried out a complete structural analysis of the antenna of *S. zeamais* using scanning (ESEM) and transmission electron microscopy (TEM), in order to characterize the olfactory sensilla equipment. In this species, there is no sexual dimorphism also as regards the antennal morphology and the sensilla type and distribution. We identified six type of sensilla, among which at least three types (Sensillum Trichoideum 1, Sensillum Trichoideum 2 and Grooved Peg Sensillum) can be considered as olfactory. EAG recordings carried out on *S. zeamais* revealed a positive dose- response to both EOs, without differences between the two sexes.

Keywords: Maize weevil, repellence, essential oli, ultrastructure, antennal sensilla, behavior, EAG

CO422

INTEGRATED PEST MANAGEMENT IN STORED PRODUCT PROTECTION

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With the phase out of methyl bromide fumigation and the increased focus on prevention in the food industry, there has been a growing emphasis on integrated pest management programs for stored-product insects in food facilities such as mills, processing plants, warehouses, and retail stores. In this presentation, I will discuss some recent research which highlights the development and improvement of tactics such as monitoring, mating disruption, aerosol applications of reduced risk insecticides, and sanitation that are contributing to more effective pest management programs. Emphasis will be placed on the importance of a sound scientific understanding of the spatial pattern of both the tactic and the target insect in achieving effective pest management in the spatially complex landscapes of food facilities. Will finish with discussion of some new next generation pest management tactics that focus on preventing insects from getting into facilities and which hold promise to further improve integrated pest management programs for the food industry.

Keywords: stored product, mating disruption, aerosol, mill, integrated pest management

CO423

KAOLIN-BASED PARTICLE FILM INTERFERES WITH GRANARY WEEVIL ORIENTATION TOWARDS WHEAT KERNELS

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Host-derived volatiles play a pivotal role in the selection of food and oviposition sites by many stored-product insect pests. The granary weevil, *Sitophilus granarius* (L.) (Coleoptera, Dryophthoridae) is one of the most damaging pest of stored cereals whose adults have been shown to be attracted by wheat kernel odours. Kaolin is a non-abrasive aluminosilicate that have been proposed as a pest management tool according to the particle film technology. The adverse effects of kaolin treatment to insect pests appeared to be caused by contact repellency, disruption of feeding and oviposition, and increased mortality. In this study, behavioural, electrophysiological, and chemical investigations were carried out in order to evaluate the effects of kaolin-based particle film on the emission of wheat kernel volatiles and the resulting olfactory response of granary weevil adults. In two-choice pitfall bioassays, the response index (RI) of adult granary weevils to kaolin-treated wheat kernels (RI=36.57) was significantly lower than that elicited by untreated wheat kernels (RI=84.36) but increased again (RI=63.79) when kaolin was removed from treated wheat kernels by sieving and exposure to air jets. This demonstrated the role of kaolin treatment in reducing kernel attractiveness. Adult weevils were significantly attracted by hexane:diethylethere (80:20) extracts of kaolin from treated wheat (RI=49.91) but not by hexane:diethylethere (80:20) extracts from kaolin alone (RI=-13.22). In the first extract, the presence of wheat kernel volatiles able to stimulate the antennal olfactory system of granary weevil adults was further confirmed by gas chromatography - mass spectrometry (GC-MS) and electroantennography (EAG), respectively. In conclusion, the study demonstrates that adsorption of host-derived volatiles by a kaolin-based particle film results in an effective disruption of insect orientation towards an attractive host substrate.

Keywords: Storage pests, inert dusts, cereal volatiles, behavioural bioassay, GC-MS, EAG

CO424

FOOD LOSS IN HISTORY: INSIGHTS INTO THE FOOD PRODUCED BUT NEVER CONSUMED

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This paper will outline part of an historical research about cereal preservation and grain losses caused by pests, with a focus on the action of insects. The research is still ongoing and has been possible first thanks to a private funding and then a MSCA grant (Horizon 2020 IF). Based on Milan and Venice archive documents, this contribution will show the impact of pests on cereals and bread supplies, against the backdrop of pre-industrial Europe. The research explores reasons, magnitude and features of food wastage in the past and gets a focus on northern Italy, although – whenever allowed – a comparative perspective will be adopted, providing insights into the whole European scenario. By ‘food loss’ literature refers to the food produced and yet turned inedible for a number of reasons: nowadays, as well as in the past, food loss accounts for a significant part of the overarching term ‘food wastage’. Essentially food loss remains distinct from ‘food waste’, since the former takes place at production, post-harvest and processing stages, whilst the latter, much more widely debated, occurs only at the end of the food chain. This study will highlight to what extent cultural aspects, such as scientific knowledge, belief system and local policies would play a role in this process. As a matter of fact, food loss is still nowadays considered as a prominent issue even in areas endowed with adequate facilities and avant-garde technologies, pointing out that innovative models for future supply chains need an holistic and creative approach to which historical knowledge can be highly beneficial. Focusing on well-documented and highly representative case-studies, this research investigates the incidence of grain losses as a whole, its main causes due to wrong practices, a still uncertain development of the entomological domain and the occurrence of strong commercial interests in urban grain supplies management. Accounting records combined with relevant scientific literature of the time will cast a light on grain losses occurring at storage and processing stages. The impact of technical aspects (such as the features of warehouses and pest-control procedures) will be widely investigated. By bringing new data on cereal preservation in a chemical-free setting, this study provides an original contribution to the current food wastage debate.

Keywords: Food Loss, Grain Storage, Urban Supplies, Pests, History

CO425

REPELLENT EFFECT OF *CASSIA FISTULA* (AMALTAS) AGAINST *TRIBOLIUM CASTANEUM*

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Bio pesticides are used to control pests, pathogen, and weeds by a variety of means. These are often considered to be important components of Integrated Pest Management (IPM) programs, and have received much practical attention as substitutes to synthetic chemical plant protection products (PPPs). Stored food grains face severe damage due to infestation by insects. The red flour beetle, *Triboliumcastaneumis* a major pest in human stored food. Seed extract from *Cassia fistula* was assessed for insecticidal and repellency properties on *Triboliumcastaneum*. Concentrations used were 10%, 5% and 2.5% of the extracts. They were tested on the adult beetle and observations were taken at 24, 48, 72 and 96 h after the treatment, the results showed that *Cassia fistula* had 71_77% repellency to *Triboliumcastaneum*. While the concentration difference have no effect on repellency.The Amaltas can be used as repellent against *Triboliumcastaneum*.

Keywords: Cassia fistula, Biopesticides, Repellency, Stored grain pests, *Triboliumcastaneum*.

CO426

COMPOSITION OF ESSENTIAL OILS EXTRACTED FROM FOUR AROMATIC PLANTS OF KABYLE ORIGIN(ALGERIA) AND THEIR BIO-ACTIVITY ON *CALLOSOBRUCHUS MACULATUS* (FABRICIUS, 1775) (COLEOPTERA: BRUCHIDAE)

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Essential oils of *Ocimum basilicum*, *Mentha spicata*, *Aloysia triphylla* and *Rosmarinus officinalis* from Kabylia were extracted and analysed by GC/MS. Their biological activity was assessed on a pest of stored products, *Callosobruchus maculatus* on cowpea (*Vigna unguiculata*).The results showed that these oils distinguishes themselves by the abundance of their various chemical constituants and sometimes even by their nature although they belong all to the same botanical family. The bio-tests were conducted through contact, fumigation and reppelency, under laboratory conditions. The essential oils toxicity acted by leading adulticide effect by Knock-down and ovicide because of the permeability of the chorion or the vitelline membrane which facilitates vapor distributions. It was noted too that 12µl/L air of all the tested oils reduced *C. maculatus* population throughout 90%.

Keywords: Key words: Essential oils, cowpea weevil, *Vigna unguiculata*, Algeria, bio-activity.

Urban and Public Health Pests

CO427

MANAGEMENT OF *THAUMETOPOEA PITYOCAMPA* (DEN. AND SCHIFF.) IN URBAN, SUBURBAN AND RECREATIONAL AREAS

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Research activities of the European Project LIFE-PISA were aimed at the development of innovative eco-friendly technologies for Integrated Pest Management of the *Thaumetopoea pityocampa* in urban, suburban and recreational areas of Greece, Italy and Spain. During 2014-2017 the following four tasks were performed. Evaluation of the effectiveness of trunk barrier trap devices on the capture of *T. pityocampa* wintering migrant larvae. Results showed a poor efficacy of the adhesive barrier devices and a high capture capacity of the commercial and LIFE-PISA prototype barrier traps. The later was installed easier and quicker than the former. Management of *T. pityocampa* infestation using trunk barrier trap devices. Effectiveness of devices was evaluated in trapping wintering migrant larvae for two consecutive years (2015 and 2016) in relation to the associated male adult population and number of winter nests on trees. Significantly fewer adult moths and larval nests were recorded after the application of the trunk barrier trap devices. Results showed the potential of a commercial trap device (Ecopiège) as a control method of *T. pityocampa*. Pheromone traps comparison in order to capture adult moths of *T. pityocampa*. From the pheromone trap devices tested, baited with 1 mg of (Z)-13-hexadecen-11-ynyl acetate, a specific prototype trap was found superior than the commercial and other prototype devices. Management of *T. pityocampa* using mating disruption (MD) technique. In each study area of Greece and Italy, two plots of one hectare were identified. There were areas that were treated with MD (gels with the pheromone of (Z)-13-hexadecen-11-ynyl acetate at 20g/ha] and other areas (as control) that were left untreated. During both years of the experiments (2015 and 2016), the numbers of the adults at the pheromone-baited traps and also nests with larvae were considerably lower in the MD-treated areas, in comparison with the controls.

Keywords: *Thaumetopoea pityocampa*, control methods, eco-friendly technologies, urbanized areas.

CO428

INSECT PESTS OF EUCALYPTUS AND THEIR IMPACT IN THE MEDITERRANEAN AREA

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Many species of Eucalyptus are of great forest and environmental interest, but they are also important in urban forestry and greening, being often present as ornamental trees in city parks, public and private gardens, campsites and holiday parks. Over the last decades, a number of phytophagous insects have threatened Eucalyptus species in Europe and, particularly, in the Mediterranean area. Among the most recent exotic invasive species, we include the psyllids *Glycaspis brimblecombei* Moore (red gum lerp psyllid) and *Blastopsylla occidentalis* Taylor (eucalyptus psyllid), and the thaumastocorid *Thaumastocoris peregrinus* Carpintero & Dellapé (bronze bug) have caused extensive damage in eucalypt plantations for consecutive years. In addition, the gall wasps *Ophelimus maskelli* Ashmead and *Leptocybe invasa* Fisher & La Salle, and less recently some beetles such as the longhorns *Phoracantha semipunctata* Fabricius and *P. recurva* Newman, and, the weevils *Gonipterus scutellatus* Gyllenhaal and Polydrusus (= Metallites) parallelus (Chevrolat) have also been reported. In recent years, the gypsy moth *Lymantria dispar* (L.) have caused defoliations in some areas. The present report contains a comprehensive review of *Eucalyptus pests* and their impact in the Mediterranean context.

Keywords: Invasive pests, psyllids, bronze bug, gall wasps, longhorns

CO429

TIME-OF-DAY SPECIFIC CHANGES IN INSECTICIDE SUSCEPTIBILITY AND METABOLIC DETOXIFICATION IN THE COMMON BED BUG, *CIMEX LECTULARIUS*

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Daily rhythms of physiological functions and behavioural patterns are generally displayed in living organisms including insects to adapt and synchronize with periodic changes in the environment. In bed bugs, studies on daily rhythms have been limited to locomotor activity and nocturnal dispersal pattern. To better understand their physiological function and as an approach to control bed bug population, we explored time-of-day specific changes in insecticide susceptibility and metabolic detoxification in the common bed bug, *Cimex lectularius*. This was done by exposing the bed bug to deltamethrin and imidacloprid at specific times across the day under 12 h: 12 h light-dark (LD) and constant dark (DD) conditions. Rhythmic expressions of glutathione S-transferase (GST), esterase and cytochrome P450 that are putatively involved in insecticide detoxification and their respective enzyme biochemical assays were also investigated in LD and DD conditions. We found that the bed bug has highest survival rate during mid-day (ZT9) in LD condition and this pattern was found repeating in DD condition suggesting influence of circadian clock regulation. One-way ANOVA revealed significant time-of-day differences in mRNA expression patterns of GST, esterase and CYP450. The observed gene expression patterns were found to be positively correlated with their respective enzyme biochemical assays in both LD and DD conditions. Similarly, we found that the chronotoxicity was associated to the gene expression as similar peak phases were observed in LD and DD conditions for GST and CYP450. This study has demonstrated rhythms in detoxification and insecticide susceptibility in *C. lectularius* and this knowledge can be utilized in devising an effective strategy of controlling bed bug population by targeting treatment during specific time of the day and contributes to our basic understanding of bed bug physiology.

Keywords: Rhythm, circadian, insecticide resistance, bed bug, *C. lectularius*, GST, CYP450, esterase

CO430

IT'S ABOUT INSTILLING CONFIDENCE: RESULTS FROM LET'S BEAT THE BUG

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Bed bugs (*Cimex lectularius*) continue to affect society and place a burden on public health systems; we present our experiences to help information networks prepare personnel to effectively address questions about this pest. Between October, 2011 and December, 2017, the Campaign received 4,627 direct inquiries and the website logged more than 2.8 Million visits. The InformationLine primarily assisted people with issues dealing with failed treatments and landlord reluctance to take effective measures against this pest. Website visits indicated a preference for learning do-it-yourself control methods. There were commonalities in the information sought from both services. Conclusion: People are often looking for reassurance, in addition to information about basic prevention and control of bed bugs. We present here priority topics that entomology staff and public health personnel should be prepared to answer if they receive inquiries about bed bugs.

Keywords: IPM, bedbugs, education, public health agency

CO431

STORED PRODUCTS' MITES: NOTES ON BIOLOGY AND PUBLIC HEALTH AWARENESS SURVEY

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Storage mites colonize and feed on food products of various kinds, such as wheat and its derivatives, cheese, salami, dry pet food, etc. Damage to foodstuffs can be caused directly or indirectly by contamination. Early detection of their presence, the species' associations and the ecological conditions for their development, implies reduction in health risks and costs. The development and survival of the Astigmata, the order of mites including most species of house dust and storage mites, are highly dependent on the conditions of environmental temperature and humidity. In general, dust mites benefit from higher temperatures (22-25°C) for development than storage mites; storage mites take advantage from conditions of high environmental humidity (>75-80% RH). In context where biological control methods are adopted, the early detection of the presence and association of different species may be critical. The simultaneous presence of different species of storage mites can determine consequences for: a) health, since these species disseminate their own allergens, and increase important cross-reactivity, as it occurs for instance among *Glycyphagus domesticus*, *Tyrophagus putrescentiae*, *Lepidoglyphus destructor*, mainly for group 2 allergens; b) modulating the control strategy according to the species present and to their biological cycle. We report experiences carried out in industry with different aging of salami, with screening and determination of species associations of storage mites and preliminary consideration for biological control.

Keywords: Storage mites, Astigmata, *Glycyphagus domesticus*, *Lepidoglyphus destructor*, food products

CO432

URBAN, STORED PRODUCT AND PUBLIC HEALTH PESTS

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Traditionally, urban, stored product and public health arthropods have been treated as distinct groups of pests. With an advent of holistic approaches for ensuring food and human environment safety, dividing pests into a particular class is (in many cases) arbitrary. Evidence is accumulating that storage pests are not only of economic but -more importantly- of medical and public health importance. Human exposure to many species of urban, storage and public health is an important asthma trigger. Different groups of insects and mites may actively or passively contribute to the dissemination of microorganisms, increasing food contamination, and they may host bacteria that have developed antibiotic resistance, contributing to their spread in food. Several emerging risks associated with storage and public heath pests have been recently identified in Europe: arthropod filth contamination of flour; mite infestation of the locally produced hams and cheeses; mite infestation of pet food; European north-south geographical shift of pasta infestation by *Sitophilus sp.* and dried fruits by mite *Carpoglyphus lactis*; unattended rodent baits as sources of insect infestation. Integrated strategies of management of the 3 pest groups may be based on both common and different tactics. The common IPM trait is a pheromone based monitoring approach, if available for a particular pest species. The control of urban and public pests mainly rely on insecticidal bait and sprays. Storage pests are mostly managed by environmental manipulations, modified atmospheres, fumigants and sprays since there is are virtually no efficient food baits available for their control. The importan emerging issue, shared among all of the three pests groups, is an incerasing physiological/behavioural resistance to pesticides and biocides that evolved for most chemical groups and formulations (*i.e.* baits, sprays and even to very toxic fumigants).

This work was partially funded by MZE-RO0418.

Keywords: pests, environment, food safety, medical effects, emerging risks, IPM

Artifact Pests

CO433

COMMERCIAL APPLICATION OF NON RESIDUAL FUMIGATION IN MUSEUMS

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Among stored product insects, non food pests are common in museums artifacts and wood products. Since the phase out of methyl bromide and since it harms rubber products non chemical fumigations were applied indoor to treat museums' valuable products such as stuffed animals, woods oriented from Far East and other valuables. Treatments were applied in controlled conditions of 25°C for 12 days to control *Lyctus spp.* and *Antrenus verbessci* beetles. The museum artifacts consisted of various stuffed wild animals collected by the Human and Animal museum, Ramat Gan and were on display inside show-window. All items were placed inside a 13m^3 gastight and flexible fumigation chamber at three levels shelves. CO^2 was applied from a pressurized cylinder using a high pressure resistant tube attached to the bottom of the fumigation chamber while at the top an opening of about 15 cm was left open. The purging of CO^2 was carried out directly using a siphon cylinder. The dosage of 2kg/m^3 was applied while gas concentration was monitored during purging and until 100% concentration was reached. Measurements were carried out immediately after application and before opening. Fumigation was done using low permeability flexible liners equipped with zip-lock zipper. During the fumigation the chamber was heated to maintain 25°C. Results obtained complete mortality of both species. CO^2 fumigation being applied indoor with controlled temperature is a suitable, successful and comfortable way of application with no need of handling the valuable, sometimes heavy, art crafts to a special gastight chamber, normally located far from museums.

Keywords: CO^2 fumigation, PE laminated envelops, museum artifacts, wood boring insects, stored products insects

CO434

ARTEFACT PESTS AND URBAN ENTOMOLOGY – DISTRIBUTION OF MUSEUM PESTS IN VIENNA, AUSTRIA

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Integrated Pest Management (IPM) is an important part of preventive conservation of museum objects made of wood, textiles, starch, paper, keratin and other organic materials. IPM is applied in many collections and museum in Europe, mainly to reduce the application of pesticides but also for a long-term protection of the objects and collections. In this presentation, long term monitoring data with sticky blunder and pheromone traps for webbing clothes moth *Tineola bisselliella* is described. What pests are the most abundant, which species are new for the indoor museum environment and when do we actually have active infestation and damage of museum objects? The analysis of the data show that in all museums and storages buildings with a monitoring in place different insect pest species are present, but only in few collections damage to museum objects was found. New pests like the grey silverfish *Ctenolepisma longicaudata* and *Ctenolepisma calva* - another species of Lepismatidae, are now found in many of our collections. The odd beetle *Thylodrias contractus* was found recently in Austria, surprisingly in four different locations across Vienna, all not related to each other.

Keywords: Pests, collection, damage, diversity, distribution

CO435

IPM IN THE HISTORICAL ARCHIVE OF THE DIOCESE OF LODI (NORTHERN ITALY)

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The Historical Archive of the Diocese of Lodi holds a documentary heritage, produced over more than a thousand years of activity, which is divided into three main sections, the oldest with about 1400 medieval parchments and more than 1700 notarial deeds, compiled from the year 883 to the 13th century. The Archive is housed in a portion of an old building in the centre of Lodi. The project, which started in 2011, aimed to introduce IPM strategy for the protection of this heritage of great historical value. First of all, the general conditions of the building were checked, including the protection of openings (windows and doors), the storage system and environmental conditions were analyzed, and the first modifications were carried out. The main substances at risk of attack by insects were paper, leather, starch and wood. Staff training and education were carried out to increase awareness and also enable them to manage a part of the IPM strategy. Data loggers were installed for recording the relative humidity and temperature of the environment. Entomological, fungal and bacterial monitoring was planned. For insects, different kinds of traps were used: pheromone traps, light traps and sticky traps for *Coleoptera Anobiidae* and *Dermestidae*, *Lepidoptera Tineidae* and *Thysanura*. The Archive staff were responsible for trap management and for recording trapping data, while Di.Pro.Ve.S. researchers were responsible for the organization of the IPM plan and for the identification of the insects trapped. The most frequently found species belong to Collembola (in a room where there was water infiltration), followed by *Lep. Tineidae*, *Thysanura*, *Col. Anobiidae*, *Ptinidae* and *Dermestidae*, *Psocoptera*. Thanks to the collaboration of all the staff, the introduction of IPM to this small site has been proved possible, simple, and above all inexpensive. The majority of the infestations were detected very early and controlled without chemical treatment.

Keywords: IPM, cultural heritage, preventive conservation, entomological monitoring

CO436

OLD BOOKS INFESTED BY *GASTRALLUS PUBENS*

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An infestation of Anobiid beetles was recently found in a library in northern Italy. Part of morphological traits did not match those of the common Anobiid species infesting books; therefore we hypothesized the presence of *Gastrallus pubens* Fairmaire (Coleoptera: Anobiidae). The deep galleries on the books were due to the action of larvae, which make tunnels in compressed paper, while new-emerged adults made circular holes, altering the readability of some pages. Most of damages were observed near the volume bounds. Aesthetic and structural damages were observed on 4.5% of books, especially the oldest one.

Keywords: Cultural heritage, book infestation, Anobiid beetles

CO437

REMARKS ON INTEGRATED PEST MANAGEMENT IN FOOD CHAINS

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A team of 17 co-Authors has published a review paper on the European Union's eight principles of Integrated Pest Management (IPM). The paper provides researchers, advisers and farmers with an approach for applying these legal requirements intelligently to promote local innovation while reducing reliance on pesticides and associated risks. The process envisioned therefore requires learning, adaptation, and tweaking of a number of farm management practices. It also requires attention to non-technical aspects such as the social environment in which farmers operate collective learning and farmer's inclination for step-wise rather than drastic changes. Also the food industry has been moving away from structural fumigations and calendar-based chemical pesticide applications towards IPM. This shift has been driven by the loss of pesticides such as methyl bromide, consumer demand for reduced pesticide usage, and development of 'precision-application' technologies and pest guidelines. These somewhat antagonistic trends (less reliance on and use of pesticides, and the demand for perfect food products) highlight one of the main challenges faced by the food industry. Food facilities typically are large, complex structures with many locations vulnerable to insect infestation. They differ from each other in function, commodity, product generated, structure type, equipment, geographic location and surrounding landscape, as well as other factors. This makes generalizations about pest management difficult. The pest situation must be characterised for a given place, and an IPM programme should be both tailored to a specific location and flexible enough to deal with changing conditions. Although pest management is part of a food facility's prerequisite programme, in many cases it can be implemented more effectively. Economic losses due to insects and unnecessary pest management expenses can be avoided using insect monitoring and decision-making tools such as economic thresholds, predictive models and expert systems to determine the best time to suppress pest populations.

Keywords: Integrated Pest Management, food industry, food chains, Europe

Session 16.
Symbiosis and Insect Vector Biology

Molecular and Cellular Interactions Between Insect Vectors and the Pathogens They Transmit

CO438

IS THE ADHESIN VMPA IMPLICATED IN THE SPECIFIC INTERACTION BETWEEN THE PHYTOPATHOGENIC FLAVESCENCE DORÉE-RELATED PHYTOPLASMAS AND THEIR LEAFHOPPER VECTORS?

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Leafhoppers, planthoppers and psyllids are vectors of phytoplasmas, uncultivated and cell wall-less bacteria that infect a wide variety of plants. Phytoplasmas of the phylogenetic sub-groups 16SrV-C and D cause a severe grapevine yellows: the flavescence dorée (FD) disease. They are transmitted from grapevine to grapevine by *Scaphoideus titanus*, introduced into Europe from North America, and can also be propagated experimentally on broad bean plants by *Euscelidius variegatus*, both leafhoppers being from the Deltocephalinae sub-family. Palatinate grapevine yellow (PGY) phytoplasmas are genetically closely related to FD phytoplasmas (16SrV-C sub-group). They can occasionally be transmitted to grapevine by leafhoppers of the Macropsinae sub-family but could not be propagated by *S. titanus* and *E. variegatus*, neither in vineyards nor experimentally from broad bean. Phytoplasmas perform a circulative and propagative cycle in insects that requires the crossing of the midgut and salivary glands. Bacterial adhesion to insect cells is a key step that possibly involves the variable membrane proteins (Vmps) exposed to the phytoplasma surface. Thus, we first demonstrate that the protein VmpA acts as an adhesin of the FD phytoplasma using VmpA-coated fluorescent beads and adhesin-deficient recombinant spiroplasmas that express VmpA proteins to their surface. Adhesion assays with *E. variegatus* cells in culture and microscopy showed that the protein VmpA specifically binds insect cells *in vitro* and the perimicrovillar membrane of the midgut. The insect protein that interacts with the protein VmpA is under identification by pull down assays followed by mass spectrometry. Finally, we demonstrated that fluorescent beads coated with the protein VmpA of phytoplasma isolate FD92 showed a better adhesion to *E. variegatus* cells in culture and a better retention in insect midgut than the fluorescent beads coated with VmpA of the phytoplasma isolate PGYA. This suggests that VmpA is implicated in the specificity of phytoplasma/insect interaction.

Keywords: Insect vector, leafhopper, phytopathogen, phytoplasma, transmission, adhesin

CO439

SUPPRESSION OF THE PELO PROTEIN BY WOLBACHIA AND ITS EFFECT ON DENGUE VIRUS IN *AEDES AEGYPTI*

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The endosymbiont Wolbachia is known to block replication of several important arboviruses, including dengue virus (DENV), in the mosquito vector *Aedes aegypti*. So far, the exact mechanism of this viral inhibition is not fully understood. A recent study in *Drosophila melanogaster* has demonstrated an interaction between the pelo gene and Drosophila C virus. In this study, we explored the possible involvement of the pelo protein, that is involved in protein translation, in Wolbachia-mediated antiviral response and mosquito-DENV interaction. We found that pelo is upregulated during DENV replication and its silencing leads to reduced DENV virion production suggesting that it facilities DENV replication. However, in the presence of Wolbachia, specifically in female mosquitoes, the pelo protein is downregulated and its subcellular localization is altered, which could contribute to reduction in DENV replication in *Ae. aegypti*. In addition, we show that the microRNA aae-miR-2940-5p, whose abundance is highly enriched in Wolbachia-infected mosquitoes, might mediate regulation of pelo. Our data reveals identification of pelo as a host factor that is positively involved in DENV replication, and its suppression in the presence of Wolbachia may contribute to virus blocking exhibited by the endosymbiont.

Keywords: Wolbachia, endosymbiont, dengue virus, mosquito, pelo

CO440

INTERACTIONS BETWEEN A CIRCULATIVE NON-PROPAGATIVE NANOVIRUS AND ITS APHID VECTOR

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The mechanisms of virus-insect vector interactions are categorized as three major transmission modes, non-circulative, circulative-propagative and circulative-non-propagative. The circulative non-propagative transmission has solely been reported for plant viruses. In this mode of transmission, the virus mandatorily cycles within its insect vector, traversing cellular barriers of the gut and salivary glands, without replicating. Virus particles are believed to pass across vector cells through transcytosis, sequestered into membranous vesicles and thus not in direct contact with the cytoplasm or nucleoplasm. However, this has been experimentally shown for the family Luteoviridae only, and the mechanisms of cell crossing for the families Gemini- and Nanoviridae remain hypothetical. In the two latter families, empirical observations are sometimes questioning the absence of viral replication in the insect cells, denoting that this mode of transmission requires increased research efforts for precisely defining the intimate relationship between viruses and their vectors. We have initiated a study on the molecular/cellular interactions between aphids and the nanovirus species Faba bean necrotic stunt virus (FBNSV). We have established the route of this multipartite virus in the aphid organs and tissues, where distinct viral genome

segments all travel together. Both other and our groups have shown the requirement of a “helper” factor for aphid-transmission of FBNSV. This helper factor is being identified as the product of the genome segment N, the protein NSP. At this point, we have evidence that the NSP protein controls the entry and/or accumulation of FBNSV into the aphid gut cells and further study of its mode of action is in progress.

Keywords: Aphids, virus, nanovirus, plant, vector-transmission, salivary glands

CO441

EPHRIN RECEPTOR PROTEIN: A NEW TARGET TO REDUCE TURNIP YELLOWS VIRUS TRANSMISSION BY MYZUS PERSICAE

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Polerovirus are a threat for major crops causing massive economic loss worldwide. Those viruses are strictly transmitted by aphids and chemicals treatments against aphids are the main way to reduce vector population and to limit virus dispersion. Nevertheless, these methods are damageable for the environment and it is crucial to elaborate alternative strategies to reduce virus transmission by vectors. Turnip yellows virus (TuYV) is a polerovirus transmitted by aphids in a circulative and non-replicative mode. Virions are acquired when aphids ingest sap from infected plants. Virus particles cross the gut epithelium and the accessory salivary gland cells before being released, together with saliva, into the plant. This highly specific transcytosis mechanism relies on the presence of virus receptors on the surface of the aphid cells. Identification of these receptors could result in the development of innovative strategies to reduce virus transmission by aphids. In the laboratory, we bring *in vivo* evidence that the membrane-bound Ephrin receptor (Eph), involved in cell communication and endocytosis in mammalian cells, is a novel aphid protein involved in TuYV transmission by *Myzus persicae*. The minor capsid protein of TuYV, essential for aphid transmission, was able to bind to the external domain of Eph in yeast. Feeding *M. persicae* on in planta- or *in vitro*-synthesized dsRNA targeting Eph-mRNA (dsRNAEph) did not affect aphid feeding behavior but reduced accumulation of TuYV genomes in the aphid’s body. Consequently, TuYV transmission efficiency by the dsRNAEph-treated aphids was reproducibly inhibited and we brought evidence that Eph is likely involved in intestinal uptake of the virion. The inhibition of virus uptake after dsRNAEph acquisition was also observed for two other poleroviruses, suggesting a broader role of Eph in polerovirus transmission. Whether Eph is responsible for virus attachment at the cell surface or virus endocytosis still needs to be elucidated.

Keywords: Polerovirus, virus transmission, virus receptor, RNA interference, transmission inhibition, plant viruses, aphid vector

CO442

PHYTOPLASMAS MODULATE INSECT VECTOR BIOLOGY

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Phytoplasmas are plant pathogenic bacteria transmitted by leafhoppers, planthoppers and psyllids. They can establish pathogenic, neutral or beneficial relationships with their vectors. The leafhopper *Euscelidius variegatus* (Hemiptera: Cicadellidae) is an efficient natural vector of chrysanthemum yellows phytoplasma (CYp) and a laboratory vector of Flavescence dorée phytoplasma (FDp). These two phylogenetically distant phytoplasmas have different effects on their common insect vector species: FDp negatively affects its fitness while CYp is not harmful. RNAseq analysis of *E. variegatus* infected with the two phytoplasmas was performed to underpin the molecular bases of these different insect responses. Among insect transcripts with a functional annotation, 97 were differentially modulated between the two conditions (CYp *vs* FDp). The differentially expressed transcripts were mainly related to immune response, movement and energy production. RT-qPCR and biological experiments were carried out to support the molecular evidences. Measures of the phenoloxidase enzymatic activity, melanization and body pigmentation indicated a stronger level of activation of the immune response in *E. variegatus* infected with FDp rather than with CYp, confirming the detrimental effect of FDp on its vector. In the immunocompetence assay *E. variegatus* displayed a prompter response to stress upon infection with CYp compared to FDp. Higher CO2 production and faster movements were recorded in CYp-infected than in FDp-infected insects. Our data show that *E. variegatus* mostly perceives FDp as a pathogen, since it activates a long-lasting immune response, probably due to the lack of co-evolution between FDp and the laboratory vector *E. variegatus*. Conversely, the acquisition of CYp, transmitted by *E. variegatus* under natural conditions, does not affect fitness of the vector, primes its defenses and improves movement of the infected insect, enhancing the opportunity of pathogen transmission and providing an ecological advantage to both the vector and the phytoplasma.

Keywords: ‘Candidatus Phytoplasma asteris’, Flavescence dorée phytoplasma, immune response, energy metabolism, leafhopper, co-evolution

CO443

BIOLOGICAL AND MOLECULAR INTERACTIONS BETWEEN THE PSYLLIDS AND LIBERIACTER

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Candidatus *Liberibacter solanacearum* (CLso) is associated with vegetative disorders in some Apiaceae species mainly carrots, and the zebra chips disease in potatoes. This bacterium is transmitted by the psyllid species *Bactericera trigonica* and *Trioza apicalis* in the Apiaceae and by *B. ckockerelli* in potatoes. Chemical sprays are currently the major methods for managing these diseases by limiting psyllid populations. A promising approach to prevent the transmission of these pathogens is to interfere with the vector-pathogen interactions, but our understanding of these processes is very limited. We have recently reported that Candidatus *Liberibacter asiaticus* (CLas), which causes the citrus greening disease, induced changes in the nuclear architecture in the midgut of its psyllid vector, the Asian citrus psyllid (ACP) *Diaphorina citri*, and activated programmed cell death (apoptosis) in this organ. CLso however, displayed different effects in the gut of *B. trigonica*, showing severe abnormalities, collapse of the nuclei and dense nuclear structures. Electron and fluorescent microscopy further showed that CLas induced the formation of Endoplasmic reticulum (ER) inclusion- and replication-like bodies. ER involvement in bacterial replication is hypothesized to be the first stage of an immune response that activate a cascade of molecular responses ending up with the observed nuclear structures. Although such ER-associated phenotypes were not observed in the CLso-*B. trigonica* pathosystem, transcriptomic analysis of the response of the psyllid gut to infection by the bacterium showed that ER-associated molecular responses are significantly induced, specifically those associated with bacterial invasion of the cell. Understanding the molecular interactions that undelay the responses to the bacteria will increase our knowledge of the psyllid-Liberibacter interactions, and will set the foundation for developing novel, and efficient strategies to disturb these interactions and inhibit the transmission.

Keywords: Psyllid, Liberibacter, vector, transmission, Molecular response, Endoplasmic reticulum

CO444

SMALL RNA PATHWAYS AND ANTIVIRAL IMMUNITY IN INSECTS

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Persistence of RNA viruses in insects requires reverse transcription of viral RNA into viral DNA. The dsRNA produced from the transcription of this viral DNA is recognized and processed by the RNAi machinery to generate siRNAs that amplify the antiviral response. We studied the specifics of protection via viral DNA molecules. We show that unintegrated circular forms of chimeric retrotransposon/viral DNA are produced after infection of Drosophila with different RNA viruses. Flies inoculated with circular viral DNA produces virus-specific siRNAs and survive longer following viral challenge. These results suggest new strategies to induce a virus-specific RNAi response and curtail arbovirus infections in insects.

Keywords: RNA viruses, Drosophila, antiviral response, virus-specific siRNAs

CO445

AEDES AEGYPTI SUMOYLATION PATHWAY SUPPRESSES ARBOVIRUS REPLICATION

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Half the world's population is at risk of infection from mosquito-borne arboviruses. The recent outbreak of Zika virus in Latin America and the Caribbean is expected to cost approximately US$18billion, demonstrating the impact of these important vector transmitted viruses. Currently, interactions between the mosquito antiviral response and infecting arboviruses remain poorly understood; deciphering these will be crucial to the development of novel methods to limit replication and transmission that could help control future outbreaks. Previous mammalian studies have shown that the *Homo sapiens* Small Ubiquitin-related Modifier (SUMO) pathway plays a fundamental role in various aspects of cell biology, including the regulation of host cell immunity. However, this pathway and its impact on arbovirus replication remains uncharacterised in mosquito hosts such as *Aedes aegypti*. Comparison between the *Ae. aegypti* and *H. sapiens* SUMOylation pathways demonstrated a high degree of amino acid sequence and structural similarity. The main predicted difference is the lack of ability of AaSUMO to form poly-SUMO chains, which have important functions in *H. sapiens*. Biochemical analysis of the core AaSUMOylation pathway identified the conserved function compared to the HsSUMOylation pathway and confirmed that AaSUMO could not efficiently form poly-SUMO chains, unlike its closest HsSUMO homologue. Catalytically inactive AaPIAS demonstrated its necessity in efficient poly-SUMO chain formation. Q-PCR investigations revealed the AaSUMOylation pathway to be ubiquitously expressed. Furthermore, confocal microscopy confirmed that AaSUMO protein is expressed in throughout the haemocytes, salivary glands, ovaries, and midgut, all of which are sites of arboviral replication. *in vitro* depletion of the AaSUMOylation pathway led to significantly enhanced levels of Zika, Semliki Forest, and Bunyamwera virus replication, identifying a vital role for AaSUMOylation in restricting the replication of these arboviruses. This is the first study to investigate the biological significance of the SUMOylation pathway in a vector species required for arbovirus transmission.

Keywords: *Aedes aegypti*, *Ae. aegypti*, Arbovirus, SUMOylation pathway, SUMO, PIAS, Protein modification

CO446

IDENTIFICATION OF PLANT VIRUS RECEPTOR CANDIDATES IN THE STYLETS OF THEIR APHID VECTORS

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Plant viruses transmitted by insects cause tremendous losses in most important crops around the world. The identification of receptors of plant viruses within their insect vectors is a key challenge to understanding the mechanisms of transmission, and offers an avenue for future alternative control strategies to limit viral spread. Most plant viruses transmitted by insect vectors bind to their mouthparts on the cuticle lining the inner face of maxillary stylets. Viruses are acquired and inoculated within seconds when insects move from plant to plant. The receptors involved remains totally elusive due to long-standing technical bottleneck in working with insect cuticle. Over the last ten years, we have discovered the acrostyle, an organ located at the distal tip of aphid maxillary stylets shown to harbor receptors of a non-circulative virus, the Cauliflower mosaic virus (CaMV). Using an antibody library specifically targeting cuticular proteins, we identified very recently the two first proteins of the acrostyle, Stylin-01 and Stylin-02. These two proteins belong to the CPR family, and are highly conserved among aphid species. We further showed that one peptide of these proteins emerges all over the surface of the acrostyle, at the virus-vector interface. We developed complementary approaches –*in vitro* competition assays and *in vivo* functional validation– to assess their involvement in the virus transmission process. Our results indicate that one of the two Stylins identified plays a role in CaMV transmission.

Keywords: Aphid, virus, receptor, cuticular protein, acrostyle, stylet

Ecology/Epidemiology/Evolution of the Three-Way Interaction Insect Vector-Pathogen-Host

CO447

DECIPHERING THE PLANT-POLEROVIRUS-APHID CROSSTALK BY A COMBINED TRANSCRIPTOMIC AND METABOLOMIC APPROACH

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Insect-vectored plant viruses represent a major threat in agriculture causing significant yield losses worldwide. To fight these crop pests, insecticides are widely applied to reduce insect populations and therefore limit virus dispersion. A better understanding of the tripartite interactions between the virus, the vector and the host plant is needed to develop innovative alternatives to these chemical treatments. Recent studies have shown that vector-borne viruses can alter the phenotype of their host plants in such ways that the behavior of their vectors is modified, thus impacting their transmission. This project explores whether an aphid-transmitted virus can manipulate the plant response to its vector in order to influence its own transmission. The pathosystem is composed of the model plant *Arabidopsis thaliana*, the Turnip yellows virus (TuYV), a polerovirus which is efficiently transmitted by the aphid *Myzus persicae* in a circulative mode. A transcriptomic analysis was conducted on TuYV-infected or non-infected plants infested for 0, 6 or 72h with non-viruliferous *M. persicae*. The samples were subjected to non-targeted and targeted metabolomic analyses. We observed a dramatic effect of virus infection on the plant's response to aphids. Indeed, most of the 3,000 genes deregulated in healthy plants after aphid infestation remained unaffected when the virus was present. Only a small proportion of deregulated transcripts were common between healthy and infected plants upon aphid infestation. We also observed that more metabolomic modifications were induced by aphids rather than by the virus. Viral infection however modified the metabolite profiles of plants infested with aphids. All together, these data indicate that TuYV-infection lowers the plant's response to aphids, which may favor virus transmission.

Keywords: Polerovirus, Aphid, Arabidopsis, Transcriptomics, Metabolomics,

CO448

DISRUPTING INSECT-MEDIATED TRANSMISSION OF PLANT VIRUSES

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Crop yields are threatened by pathogens, of which plant viruses represent the major emerging group. Many viruses are vectored by hemipteran insects (aphids, whiteflies) that feed using specialised piercing mouthparts called stylets. In certain hosts such as *Arabidopsis thaliana* (Col-0) the aphid-transmitted virus cucumber mosaic virus (CMV) induces aphid feeding deterrence that inhibits settling of the aphids and encourages them to move to other hosts promoting virus transmission (Westwood *et al.* 2013). We investigated the effects of CMV infection on host-vector interactions in various Arabidopsis accessions. Aphid growth rate on CMV infected Arabidopsis accessions displays a continuum of responses from aphid resistance to aphid susceptibility. The innate attraction of aphids towards certain hosts can be used to manipulate virus-vector-host dynamics to decrease virus spread. We screened for Arabidopsis accessions that were aphid attractive hosts to be engineered as virus resistant plants using a hairpin RNA construct targeting the 2b CMV region. Using Arabidopsis 'microcosms', we evaluated the effects of mixing various proportions of aphid attractive and less attractive plants, as well as virus-resistant plants, on aphid-mediated transmission of viruses. We found that mixing specific proportions of virus resistant plants and susceptible plants disrupted transmission of CMV by aphids. Thus, virus-vector-host interactions can be manipulated to limit aphid mediated virus spread.

Keywords: plant virus, *Myzus persicae*, Arabidopsis, insect vector, CMV

CO449

INSIGHTS INTO THE TRANSMISSION DYNAMICS OF BEETLE-BORNE VIRUSES

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Beetle-borne viruses have a unique mode of transmission, though their interactions with host plants and vectors remain poorly understood. Mexican bean beetles (*Epilachna varivestis*) are efficient vectors of several soybean-infecting viruses, including Bean pod mottle virus (BPMV). In these studies, we describe how BPMV-induced changes to plant physiology alter beetle feeding preferences in a way that likely facilitates virus spread. In addition, we describe the composition of oral secretions that feeding beetles regurgitate onto host plants, and how these secretions may play a fundamental role in beetle-borne virus specificity.

Keywords: Beetles, Viruses, Transmission

CO450

TRANSMISSION OF THE CODIRO STRAIN OF *XYLELLA FASTIDIOSA* BY DIFFERENT INSECT SPECIES

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Most of the diseases associated to the insect-transmitted bacterium *Xylella fastidiosa* (Xf) have been described in North and South America, but in the last five years widespread infections were reported in a restricted area of the Apulia region (southern Italy), on olives suffering a severe disease denoted "olive quick decline syndrome - OQDS". Following this first report, several outbreaks have been reported in Corsica and Balearic Islands, and some in mainland France and Spain. Because many xylem sap-feeding insects can function as vectors for the transmission of this exotic pathogen in EU, several research programs are ongoing to assess the role of candidate vectors in the spread of the infections. Initial investigations identified *Philaenus spumarius* as the predominant vector species in the olive orchards affected by the OQDS. Additional experiments carried out over the past two years included, the spittlebugs *Philaenus italosignus* and *Neophilaenus campestris*, and of the planthopper *Latilica tunetana* (Hemiptera Issidae). Adults have been tested in transmission experiments to assess their ability to acquire the bacterium from infected olives and to infect different susceptible plant species. Acquisition rates determined by testing individual insects in quantitative PCR assays, ranged from 4,9% in *N. campestris* to 22% in *P. italosignus*, whereas no acquisition was recorded for *L. tunetana*. Successful transmissions were detected in some of the plant species exposed to *P. italosignus* or *N. campestris*, whereas *L. tunetana* did not transmit, even when adults were collected directly from the canopies of field-infected trees and tested positive in qPCR (1,4%). The ecological and epidemiological relevance of these findings are discussed. Although still limited, the data so far collected on the candidate vectors of Xf in Europe remark the major, if not exclusive, role of spittlebugs in the spread of this emerging pathogen.

Keywords: Spittlebugs, *Philaenus italosignus*, *Neophilaenus campestris*, Aphrophoridae, OQDS

CO451

VECTORS OF XF IN EUROPE: BEHAVIORAL ASPECTS INVOLVED IN TRANSMISSION

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Vector-borne plant pathogens spread is mediated by a continuum of interactions among three main components: the pathogen, the host, and the vector. Considering the bacterium *Xylella fastidiosa*, one of the most frightful pathogens threatening Europe, many steps have been done toward the direction of a complete understanding of the pathosystem's components and reciprocal interactions. Nevertheless, despite decades of efforts, a fundamental question remains unsolved: which specific vector behavior is associated with bacterium transmission? Previous research definitely proved that *X. fastidiosa* transmission is a mechanism driven by an active vector behavior, such as the previously proposed salivation-egestion hypothesis. However, to date conclusive evidences about salivation-egestion and its possible involvement in bacterial cells delivery to the plant are still missing. Moreover, the applicability in the European scenario of the background about *X. fastidiosa* transmission is hampered by the fact that all the previous studies were carried out with sharpshooters (Hemiptera: Cicadellidae), the main American vectors. On the other side, the spittlebug *Philaenus spumarius* (Hemiptera: Aphrophoridae), to date considered the most important European vector of the bacterium, has shown elements of novelty compared to sharpshooter in respect to its relationship with the bacterium. Therefore, part of the outline valid for sharshooters should be questioned and further validated for those vector families widespread in Europe, and disregarded in previously studied outbreaks, such as spittlebugs and cicadas. Here we briefly review evidences and theories about the mechanism related to *X. fastidiosa* transmission, with a special focus on *P. spumarius* feeding behavior and its likely role in the epidemiology of *X. fastidiosa*-related diseases in Europe.

Keywords: *Xylella fastidiosa*, vectors, transmission, EPG

CO452

PHYTOPLASMA VIRULENCE PROTEINS ALTER PLANT DEVELOPMENT AND PROMOTE COLONIZATION OF INSECT VECTORS

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One of the most spectacular phenomena in biology is the complete hijacking of hosts by parasites such that these hosts become 'zombies' destined to advance survival of the parasites often to the detriment of the hosts. Phytoplasmas are obligate intracellular bacterial parasites of plants that induce dramatic changes in plant development, including proliferation of stems (witch's brooms) and the reversion of flowers into leaf-like structures (phyllody). These bacterial parasites produce two virulence proteins (effectors), named SAP11 and SAP54, that promote the degradation of plant TCP and MADS-box transcription factors, respectively, thus altering leaf and flower development. Phytoplasmas are dependent on sap-feeding insects for transmission to plants. Interestingly, SAP11 and SAP54 also convert plants into more attractive hosts for egg laying and reproduction of the insect vectors. Thus, phytoplasma effectors interfere with key plant developmental processes. Moreover, these effectors increase the colonization of plants by insect vectors thereby promoting transmission of the obligate phytoplasmas to new plant hosts.

Keywords: Symbiont, symbiosis, bacteria, phytoplasma, Hemiptera, pathogen, insect vector, leafhopper, aphid, virulence, effector, molecular mechanism, functional genomics, metagenomics, insect behavior, plant defense, insect-plant interactions, insect-microbe interactions, plant-microbe interactions, plant development, transcription factor, parasite

CO453

EVOLUTIONARY DETERMINANTS OF HOST AND VECTOR MANIPULATION BY PLANT VIRUSES

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Plant viruses possess adaptations for facilitating acquisition, retention, and inoculation by vectors. Until recently, it was hypothesized that these adaptations are limited to virus proteins that enable virions to bind to vector mouthparts or invade their internal tissues. However, increasing evidence suggests that viruses can also manipulate host plant phenotypes and vector behaviors in ways that enhance their own transmission. Manipulation of vector-host interactions occurs through virus effects on host cues that mediate vector orientation, feeding, and dispersal behaviors, and thereby, the probability of virus transmission. Effects on host phenotypes vary by pathosystem but show a remarkable degree of convergence among unrelated viruses whose transmission is favored by the same vector behaviors. Convergence based on transmission mechanism, rather than phylogeny, supports the hypothesis that virus effects are adaptive and not just by-products of infection. Based on this, it has been proposed that viruses manipulate hosts through multifunctional proteins that facilitate exploitation of host resources and elicitation of specific changes in host phenotypes. But this proposition is rarely discussed in the context of the numerous constraints on virus evolution imposed by molecular and environmental factors, which figure prominently in research on virus-host interactions not dealing with host manipulation. To explore the implications of this oversight, we synthesized available literature to identify patterns in virus effects among pathogens with shared transmission mechanisms and discussed the results of this synthesis in the context of molecular and environmental constraints on virus evolution, limitations of existing studies, and prospects for future research.

Keywords: Host phenotype manipulation, vector behavior, disease ecology, chemical ecology, virus evolution, fitness, adaptation, generalist *vs* specialist, host diversity, landscape heterogeneity

CO454

SENSORY STRUCTURE OF *PHILAENUS SPUMARIUS* (L.) MOUTHPARTS AND OPERATIVE MECHANISM OF THE PRECIBARIAL VALVE

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In phytophagous species, insect mouthparts host important sensory structures involved in the plant selection and acceptance. In several species belonging to Homoptera, a frequent rubbing or tapping behaviour has been reported, performed with the tip of the labium and preceding the insertion of the stylets. It was also observed that stimuli perceived through precibarial sensilla play an important role in host discrimination. The morphology and the ultrastructure of the labial tip and precibarial sensilla of *Philaenus spumarius*, the main vector of *Xylella fastidiosa* in Italy, was examined using scanning and transmission electron microscopy techniques. The labium tip bears 5 different type of sensilla: trichodea type 1 and 2, aporous; chaetica type 1 and 2, uniporous; and basiconica, multiporous. The precibarium, on the epipharinx, presents 18 sensilla separated in two groups, divided by the precibarial valve. The first group, distally located, is composed by 10 oval papillae sensilla. The second group, prossimally located, is composed by 6 papillae sensilla and two large bulbous sensilla. On hypopharynx two papillae sensilla are present. The relationship between all of these structures and feeding by the insect is detailed in a feeding mechanism hypothesis. The functional anatomy of the valve was examined using light and electron microscopy. It shows a bell-like structure, previously undescribed, that opens by a dilator muscles and closes through cuticular and fluid tensions. The operative mechanism of the valve is proposed.

CO455

OMICS DISSECTION OF VECTOR-PATHOGEN INTERACTION OPENS NEW PERSPECTIVES FOR THE BIOLOGICAL CONTROL OF PHYTOPLASMA DISEASES

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Hemipteran-vectored diseases are detrimental to many crops causing devastating effects on food security. However, the mechanisms behind the complex multi-trophic interactions between insect hosts and their microbiota, especially regarding the establishment and transmission of vector-borne microbial pathogens affecting plant health, are still widely unexplored. We have investigated this complex interplay using as a model the economically important apple proliferation disease that is caused by the bacterium Candidatus *Phytoplasma mali* and vectored by psyllids of the genus *Cacopsylla*. Our sex-specific multi-omics analysis, combining transcriptomics, metabolomics and metagenomics, unveiled that the presence of the pathogen is related to significant changes in the microbial community composition and seems to influence on the insect's sugar metabolism, signal transduction, rhythmic processes, cell differentiation and reproduction. Our study sheds light on the dynamic and sex-specific interactions between psyllids, their associated microbes and vectored pathogens, thus furthering a sustainable disease management of vector-borne plant disease.

Impact of Symbionts on Insect Biology

CO456

THE RICKETTSIA BACTERIAL SYMBIONT MODIFIES PLANT RESISTANCE AND WHITEFLY VECTOR EFFICIENCY

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Vertically acquired, endosymbiotic bacteria such as those belonging to the Rickettsiales are known to influence the biology of their arthropod hosts by manipulating morphological, developmental and physiological traits in ways that maximize their spread. For example, the Rickettsia symbiont can provide strong fitness benefits to its insect host, *Bemisia tabaci*, and infected whiteflies quickly outcompete their uninfected counterparts. In this study, we investigated two potential routes by which the Rickettsia endosymbiont could enhance whitefly host fitness: direct effects on the whiteflies and indirect effects via modification of host plant resistance. To confirm the previously reported effects of symbiont infection on whitefly fitness, we evaluated life-cycle metrics of Rickettsia-infected or non-infected *B. tabaci* on resistant or highly susceptible host-plants. To determine if these effects are mediated through differential manipulation of host quality by infected *vs* uninfected *B. tabaci*, we evaluated life-cycle metrics of non-infected *B. tabaci* on plants previously damaged by conspecifics with and without Rickettsia infection, and on plants with no prior damage. To determine if Rickettsia infection has direct effects on whitefly feeding behaviors associated with host plant exploitation and nutrient intake rate, we monitored individual stylet behaviors of infected and uninfected *B. tabaci* using the electrical penetration graphing technique (EPG). We also explored the influence of Rickettsia-mediated behavioral modifications on whitefly transmission of a semi-persistently transmitted plant virus. Our results suggest that the two mechanisms, direct and host-mediated, are involved as i) Rickettsia infection in the attacking whiteflies enhances their performance on both highly resistant and susceptible host plants, and ii) plant pre-infestation by symbiont-carrying whiteflies also enhances host-plant quality for non-infected whiteflies feeding in a different location. Direct effects of Rickettsia infection on feeding behavior were subtle but may also contribute to the enhanced fitness of symbiont-infected *B. tabaci*, especially on more resistant hosts.

Keywords: Endosymbiont, whitefly, vector, fitness, feeding behavior

CO457

THE MICROBIAL NETWORK IN THE INSECT METAORGANISM

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In the last few tens of years the study of plant and animal biology has been enriched with the metaorganism concept. It is now clear that plants and animals are associated to defined groups of microorganisms that affect a wide range of biological features of the host. The arthropod metaorganism and in particular the insect metaorganism played and are currently playing a major role for the understanding of the relationships between the host and the microbial partners. The host-symbiont functional interactions have been initially associated to the beneficial effects of the microbial partners on the host nutrition, health and resilience from stresses. But it was later discovered that the interaction can be much more intimate, with microbial symbionts contributing to regulate the host sexuality and behavior and even its development. While the microbial partners essential, or highly desirable, for a normal insect life have been revealed to belong to a relatively restricted number of microbial groups and have been well studied, the vast majority of the very diverse microorganisms to which most of the insect hosts are exposed to, the so called commensals, have been largely neglected. However, such a large range of microorganisms is invariably associated to all of the host individuals, where they contribute to the establishment of complex microbial networks. In this lecture I discuss the insect metaorganism concept beyond the role of specific, more or less essential, symbionts, but in the light of the microbial networks and the tails of the diversity distribution invariably associated to any insect host. I will take advantage of examples of specific insect metaorganism models to highlight the potential role of such neglected commensals and their possible exploitation for insect management.

Keywords: Insects, microbial symbionts, metaorganism, microbial networks, rare taxa

CO458

REDUCED PHYTOPLASMA ACQUISITION BY LEAFHOPPERS AFTER INFECTION BY SYMBIOTIC ASAIA

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In insect vectors of microbial pathogens, infection by facultative symbionts may alter the transmission process, as many symbionts are able to compete in colonizing organs, express antagonistic factors or activate host immune response. A major group of insect-vectored plant pathogens is that of phytoplasmas, which colonize plant phloem and are transmitted by phloem-feeding Hemiptera such as leafhoppers, planthoppers, and psyllids. In *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae), the vector of Flavescence dorée phytoplasma (FDP) to grapevine, acetic acid bacteria of the genus *Asaia* are dominant symbionts previously proposed as potential symbiotic control agents against phytoplasmas. To test this hypothesis, we experimentally investigated the interaction between different strains of Asaia and FDP transmission in the laboratory, by using the model cicadellid *Euscelidius variegatus* Kirschbaum and the plant host *Vicia faba* L. Our laboratory populations of *E. variegatus* hosted in low concentration an uncultivable strain of Asaia; moreover different cultivable exogenous strains, exhibiting different phenotypes, were orally supplied to nymphs of the leafhopper. These strains were able to stably colonize the insect host, reach high densities and be reisolated from the leafhopper. Asaia-colonized *E. variegatus* nymphs were then used for phytoplasma transmission trials. Leafhoppers that were colonized by one of Asaia strains producing an air-liquid interface biofilm exhibited significantly reduced phytoplasma acquisition compared to control insects never fed in the presence of any exogenous Asaia: infection rates in *E. variegatus* fed with Asaia were 5-28%, whereas they were 25-77% in control insects. Nevertheless, in those individuals where the pathogen succeeded in insect infection, phytoplasmas were transmitted with similar efficiencies in Asaia-treated leafhoppers and control specimens. The mechanisms regulating the reduction of phytoplasma acquisition remain to be elucidated, as well as its effectiveness in *S. titanus*. However, our results provide evidence of the potential use of Asaia as a biocontrol agent.

Keywords: Flavescence dorée, Acetic acid bacteria, vector competence, symbiotic control

CO459

INVESTIGATING WOLBACHIA-MEDIATED ARBOVIRUS INHIBITION

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Wolbachia are bacterial endosymbionts found in a broad range of arthropods, including some species of mosquitoes. They are maternally transmitted via the ovaries and manipulate host reproduction to ensure their propagation throughout a population. Wolbachia are naturally found in *Aedes albopictus*, but not the closely related *Aedes aegypti*, the primary vector of Dengue virus. When Wolbachia is artificially transinfected into *Ae. aegypti* there is a pathogen-blocking phenotype, limiting the transmission of Dengue, Chikungunya and Zika virus. Wolbachia-infected *Ae. aegypti* are currently being implemented as a vector control strategy in the wild, yet the precise mechanism of pathogen-blocking is unknown. Pan *et al.* (2012) showed higher levels of Reactive Oxygen Species (ROS) in Wolbachia-infected mosquitoes which activated the Toll immune pathway, resulting in Dengue inhibition. However, others have shown immune upregulation is not required for pathogen blocking, therefore the role of ROS in Wolbachia-mediated inhibition is ambiguous. We investigated the role of Reactive Oxygen Species (ROS) in Wolbachia-mediated pathogen blocking and subsequent interference this may have with viral replication. Results suggest Wolbachia-infected *Ae. aegypti* (Aag2) cells have higher levels of ROS, but similar levels of lipid peroxidation. We found reduced Xbp1 splicing and Calnexin levels with Wolbachia infection, which are involved in endoplasmic reticulum (ER) homeostasis. These data suggest Wolbachia may be interfering with the ER which may be preventing viruses from using this crucial structure for replication.

Keywords: Endosymbiont, mosquito, wolbachia, arbovirus

CO460

THE SPERM PRECEDENCE PATTERN OF SPIDER MITES EVOLVES IN RESPONSE TO COSTS ASSOCIATED WITH THE PRESENCE OF WOLBACHIA

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In spider mites, only the first mating of a female is effective. Still, males often copulate with mated females. This behaviour may be adaptive if it decreases the costs of incompatible crosses that occur, for example, when uninfected females mate with males infected by Wolbachia, an endosymbiotic bacteria. Such crosses result into cytoplasmic incompatibility (CI), entailing the embryonic death of fertilized offspring. Wolbachia-uninfected females are thus expected to evolve strategies to avoid the costs of CI. For example, they may mate multiply to recover offspring viability. However, such recovery may not be effective in species with first male precedence and its occurrence may be contingent upon infection history. To test this, we performed experimental evolution of spider-mite populations that were infected or uninfected by Wolbachia (controls), or in which Wolbachia-uninfected females were given the choice between Wolbachia-infected and Wolbachia-uninfected males at each generation (selection treatment). After 20 generations, Wolbachia-uninfected females from the selected treatment reduced the degree of CI by mating with an uninfected male after mating with an infected male. This was not the case when Wolbachia-uninfected individuals were from control populations. Our results suggest that the evolution of CI-reducing strategies is contingent upon the evolutionary history of infection.

Keywords: Symbionts; mating systems; experimental evolution; cytoplasmic incompatibility

CO461

WOLBACHIA AND PLANT PATHOGEN MUTUAL EXCLUSION: A CASE STUDY

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The maternally transmitted α-proteobacterium Wolbachia pipientis is commonly known as a “reproductive parasite” of arthropods and mainly insects where it has been estimated that about 76% of the species can harbour the symbiont. Although the infection is usually pervasive in populations, it was argued that vertical transmission alone does not explain the large distribution of the bacterium among arthropods. Hence, horizontal transmission was assumed as a possible mechanism promoting the spread of the symbiont among taxa of related organisms, as well as among those showing close relationships, like prey-predator, parasite-host, and parasitoid-hosts, or among species of herbivorous insects that acquire Wolbachia by ingesting tissues of the host plants contaminated with the bacteria. In this case the transmission route of the bacterium resembles that of plant pathogens. In insects, Wolbachia is able to induce feminization of the host genotypic males, parthenogenesis, male killing and cytoplasmic incompatibility: these modifications of the host breed impart a selective advantage which allows Wolbachia to spread efficiently into the host populations. On the other side, several cases of positive effects induced by Wolbachia in the host fitness have been described, including protection against viruses, thus conferring evolutionary/adaptive advantages to the host. If generalized, the antiviral protection associated with Wolbachia infection might be exploited in future strategies to reduce insect-transmitted diseases. Besides viruses, this protection has been well documented for protozoans, filarial nematodes, as well as some bacterial species. In addition, mutual exclusion or competition between Wolbachia and other endocellular symbionts has been suggested. Here we provide evidences on a natural Wolbachia infection in a phytophagous insect species where the symbiont induces feminization of genetical males and mutual exclusion of endosymbiotic plant pathogens that have been detected in the uninfected populations. The exploitation of Wolbachia as a biocontrol agent preventing plant pathogens spreading is discussed.

Keywords: Feminization, phytophagous insects, plant pathogens, Wolbachia

CO462

THE INTRAMITOCHONDRIAL BACTERIUM MIDICHLORIA, MUTUALIST OF TICKS, VECTOR-BORNE AGENT OR BOTH?

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Obligate haematophagy is a fundamental trait in tick (Acari:Ixodida) biology. Blood feeding, makes them important pathogen vectors worldwide, but also represents an abundant, yet incomplete, nutrient. Both aspects must be considered when investigating the role of bacteria associated to ticks. *Midichloria mitochondrii* is an intracellular bacterium, member of the order Rickettsiales, that has been first detected in association with the tick *Ixodes ricinus*, at very high prevalence. Midichloria presents the striking capability to colonize host mitochondria. The role of Midichloria in the biology of the tick host is currently unclear, is it a mutualistic symbiont? Is it a vector-borne agent? A MultiLocus Sequence Typing approach was performed, investigating in parallel the population structures of *I. ricinus* and of the symbiont. Very low genetic variability in the Midichloria population was detected, with no supported population structure. This trait is suggestive of a recent sweep in the host population, driven by strong selective pressure, horizontal transfer, or both. The genome of Midichloria encodes genes for B vitamins biosynthesis, suggesting that the bacterium is a mutualist that provides the host with vitamins, essential for the nutrition and fecundity of ticks, that are lacking in the blood meal. Such a role, proven for other symbionts of blood feeders, could explain the high prevalence and the low genetic variability. On the other hand, Midichloria is also widespread in other tick species, at variable prevalence levels, with no evidence of co-cladogenesis. These traits suggest horizontal transmission. Investigating the presence of Midichloria in the blood of parasitized mammals, both DNA and antibodies against the bacterium were found. Midichloria is thus not only a symbiont, but is also inoculated to the tick hosts. Midichloria could be providing an advantage to ticks, while using them as vehicle for transmission.

Keywords: Symbiosis, ticks, midichloria, vector-borne agents

CO463

BEYOND NUTRITION: HOST-MICROBIOTA INTERACTIONS DRIVE SHIFTS IN THE BEHAVIOURAL PHENOTYPES OF COCKROACHES

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Recent studies have shown that host-microbiota interactions can lead to dramatic changes in host phenotype especially behaviour. We investigate the causal drivers of microbe-associated shifts in host phenotype by examining how feeding behaviour is modulated by a bacterial infection in an omnivorous cockroach: *Blatta orientalis*. We conducted food-choice experiments after challenging hosts with the common entomopathogenic soil bacterium *Pseudomonas entomophila* to understand the impact of bacterial pathogens on host macronutrient preference. We find that immune challenge by this bacterium drives a sharp decline in carbohydrate intake and results in a relative increase in the ratio of protein to carbohydrate consumed. Additionally, infected cockroaches reduce their overall nutrient intake. We show for the first time that cockroach feeding behaviour is dynamically modulated by a pathogen. In contrast to studies on Spodoptera moths, this modulation does not impact any of the immune parameters

we measured: it does not affect the abundance of immune related proteins in the hemolymph, hemolymph antimicrobial activity, or survival. This leads to the possibility that as long-lived omnivorous species *B. orientalis* may be better adapted to unpredictable variation in food availability and quality. An illness-induced anorexia-like response which is thought to assist hosts in limiting nutritional resources available to pathogens is therefore the most likely cause of the observed phenotype. Reduction of carbohydrate intake by sick individuals would be consistent with such an explanation. In another cockroach species *Blatella germanica* we also investigate whether in addition to pathogens, host microbiota especially gut commensals are causally responsible for shifts in host behavioural phenotype and immune competence. To address this, we conduct food-choice and immune-challenge experiments in germ-free cockroaches and naïve cockroaches.

Keywords: bacterial infection, feeding behaviour, gut microbiota, illness-induced anorexia, proteomics”

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Posters

Session 1.
Agricultural and Forest Entomology

Invasive Species Biology, Ecology and Management

PO001

PRELIMINARY EVALUATION OF *CANNABIS SATIVA* AND *LAVANDULA ANGUSTIFOLIA* BIOACTIVITY AGAINST *DROSOPHILA SUZUKII* (DIPTERA: DROSOPHILIDAE)

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Cannabis sativa L. or hemp is one of the oldest known medicinal plant that had been cultivated for centuries for their seeds and fiber. While *Lavandula angustifolia* L., known as lavender, is an aromatic plant and its essential oil was found to have medicinal, antibacterial, antifungal and insecticidal activities. The goals of this study were to evaluate the anti-oviposition activities of lavender essential oil and flowers, leaves and roots extracts of hemp against *Drosophila suzukii* Matsumura (Diptera, Drosophilidae) female flies. The study was carried out by using a two-choice bioassay where *D. suzukii* female flies (n=10) were inserted into a plastic container (21x30x11 cm) and were exposed to a standard artificial medium (control) with three different doses of hemp extracts (1X, 2X, 4X) and two different doses of lavender essential oil (0.1 and 1 ml/l). Number of eggs laid in treatment and control was counted after 24 h. Our results showed that number of eggs laid in medium with flowers, leaves (lower doses) and roots (higher doses) hemp extracts was significantly higher ($P > 0,05$; test-t) than control. Oviposition activity of *D. suzukii* was inhibited at the highest dose of lavender essential oil, while at the lower dose the mean number of eggs laid was lower than control, but not significantly different ($P > 0,05$; test-t). These preliminary results showed that hemp extract would seem attractive for ovideponent *D. suzukii* females, but it appears that root extract have good insecticidal activity against flies. In fact, from the eggs laid on medium with root extracts a limited number of individuals were developed. *L. angustifolia* essential oil showed a good anti-oviposition rate, which can indicate that it has a contribution in the control strategies against *D. suzukii*. Further studies are needed to confirm these preliminary results.

PO002

OVERWINTERING CAPABILITY OF *TRICHOPRIA DROSOPHILAE* A POTENTIAL BIOLOGICAL CONTROL AGENT OF *DROSOPHILA SUZUKII*

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Trichopria drosophilae (Perkins, 1910) (Hymenoptera: Diapriidae) is an endoparasitoid of *Drosophila* spp. pupae recorded from several parts of the world. It is currently considered for augmentative biological programs to control the invasive agricultural pest *Drosophila suzukii* (Matsumura, 1931). To evaluate its establishment potential in temperate regions, we are investigating the low temperature tolerance of *T. drosophilae*. A colony of *T. drosophilae* was established from individuals captured in 2017 in Zürich, and characterized under laboratory and semi-field conditions. Super cooling points of unfed 2-day-old adult *T. drosophilae* were determined by cooling specimens down at a rate of ~1.0°C/ min and measuring the release of latent heat with type-T copper-constantan thermocouples. To assess cold hardiness, 2-day-old individuals emerged from three different drosophilid hosts: *D. suzukii*, *D. melanogaster*, and *D. subobscura* were exposed for varying durations (from 1 h to 11 d) to constant low temperatures of 0, -5, and -10°C. Prior and subsequent to the exposure, *T. drosophilae* were acclimatized for 6 h at 10°C for prohibiting cold stress. Survival and reproduction ability were recorded after cold exposure. Adults were able to survive and reproduce after short periods of extremely low temperatures but long term exposure to temperatures below 0°C was lethal after a maximum of 11 days. The implications of these findings as well as the influence of host species and parasitoid life stage on survival under laboratory and field conditions will be discussed.

Keywords: Invasive species, Spotted Wing Drosophila, Parasitoid, Super Cooling Point, Cold Tolerance, Hibernation

PO003

ABUNDANCE OF THE THRIPS SPECIES ON VARIOUS CITRUS VARIETY IN ADANA PROVINCE, TURKEY

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Thrips hawaiiensis (Morgan) (Thysanoptera: Thripidae) was the first time reported in Turkey and it spread quickly over the region. This thrips cause serious damage on young lemon fruits by scarring and bronzing particularly in lemons which flowering a year round such as Kutdiken lemon variety. After introduce of the invasive thrips species *T. hawaiiensis* in Turkey, thrips fauna and their economic importance is not clearly known. For this aim, thrips abundance was investigated on various citrus variety namely Dobashi beni, Ortanique, W Murcott mandarins, Eureka, Meyer and Kutdiken lemons and Washington Orange in 2017 in Adana, Province, Turkey. Experiments were carried out in the commercial citrus orchards of the Research and Implementation Area of Agricultural Faculty, University of Çukurova located at Balcalı district, Adana province. Each citrus variety has been grown in the area with 5 ha. 6 citrus trees of each citrus variety were randomly selected and one inflorescence representing each four cardinal directions of each tree, totally 24 inflorescences were tapped on the white plastic container for 5-10 seconds. A total of 14 thrips species were identified on citrus flowers. *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (3651 specimens) was the most common and frequently sampled from the flowers of all citrus varieties. *Thrips hawaiiensis* was the second more common thrips species, representing 298 individuals. Total individual numbers of other insect species ranged from 1 to 5. Citrus variety was an important factor influencing abundance of each thrips species. Most of *F. occidentalis*, *T. tabaci* and *T. hawaiiensis* were extracted from flowers of Meyer lemon having multi flowers and flowering through second week of May. In general, major thrips species showed main population increases in mid-April but densities of *T. hawaiiensis* were greater on flowers of Meyer and Washington varieties at the first week of May in comparison to densities of other two thrips species. No thrips damage was detected sampled young fruits of all citrus varieties. It is concluded that thrips are not recognized as harmful insect on the various citrus varieties in the location.

Keywords: *Thrips hawaiiensis*, abundance, citrus, Adana, Turkey

PO004

PHYTOPHAGOUS INSECTS OCCURRING ON MAIZE (*ZEA MAYS* L.) IN POLAND FROM THE MIDDLE OF THE 20TH TO THE BEGINNING OF THE 21ST CENTURY

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Maize (*Zea mays* L.) is one of the most important crops in Poland. Among pests, phytophagous insects impact both the quality and quality of crop yields. Research on them has been carried out in Poland since the 1950s of the 20th century. In the middle of the 20th century on the maize acreage in Poland 32 species of the phytophagous insects were identified, belonging to 7 main orders: Lepidoptera, Coleoptera, Hemiptera, Diptera, Hymenoptera, Orthoptera and Thysanoptera. At that time the most serious insect pests of maize were 4 species: *Ostrinia nubilalis* Hbn., *Agriotes ustulatus* Schall., *Selatosomus latus* F. and *Oscinella frit* L. Despite their smaller number, important pest were also: *Agrotis segetum* Den. & Schiff., *Melolontha hippocastani* F., *Melolontha melolontha* L. and *Gryllotalpa gryllotalpa* L. At the beginning of the 21st century, with the increase of the maize growing area in Poland to more than a million hectares, widespread simplifications in the agrotechnical procedures (long-term monocultures) as well as observed climate changes, a significant increase in the numer of phytophagous insects was stated. In comparison with the 1950s of the 20th century their numer had trippled. Nowadays on the maize crops the presence of over eighty pest species is noted, and economic losses are caused mainly by: *Ostrinia nubilalis*, *Agrotis segetum*, *Lacanobia oleracea* L., *Agrioties lineatus* L., *Agrioties obscurus* L., *Diabrotica virgifera virgifera* LeConte, *Melolontha melolontha*, *Phyllopertha horticola* L., *Metopolophium dirhodum* Walk., *Rhopalosiphum padi* L., *Oscinella frit*, *Anaphothrips obscurus* Müller, *Frankliniella tenuicornis* Uzel, *Haplothrips aculeatus* F., *Haplothrips tritici* Kurdjumov, *Limothrips denticornis* Haliday and *Thrips flavus* Schrank.

Keywords: Maize, insect pests, species composition, Poland

PO005

INTERACTIONS BETWEEN *DRYOCOSMUS KURIPHILUS* AND ITS ASSOCIATED NATIVE PARASITOIDS IN THE NORTHWEST OF SPAIN

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The Asian chestnut gall wasp (ACGW) *Dryocosmus kuriphilus* has become one of the major threats to the *Castanea sativa* trees in most European countries, and also in Spain. In Galicia (northwestern Spain), the area with highest chestnut production of the country, it was firstly detected in 2014, being currently spread throughout the region. The interactions among ACGW, the galls that induced and its natural enemies are not completely understood, although they are important for the development of effective management strategies against this pest. The objectives of the present study were: (i) to identify ACGW native parasitoids; (ii) to estimate parasitism rate and its relation with gall characteristics (type, size and gall toughness) and the time of arrival of ACGW. On spring and summer of 2017, 8.678 ACGW galls were collected on 102 sites throughout the region. Galls were arranged in rearing cages, and the emerged parasitoids wasps were identified. Additionally, 90 galls (30 per gall type: on leaves, leaf stipules and buds) were collected weekly from four different sites with different time of invasion. We measured size and toughness of each gall and subsequently galls were dissected to assess parasitism rates. Sixteen species of native parasitoids were identified. Average parasitism rate was 17.2%, being significant lower in buds galls (13.0%) than on leaves (18.2%) and stipules (20.3%), which can be related with the significantly higher toughness of bud galls. Parasitism rates vary through the region, depending on the ACGW arrival time.

Keywords: *Dryocosmus kuriphilus*, invasive pest, chestnut, biological control, Iberian Peninsula

PO006

ELECTRON BEAM-INDUCED STERILITY AND INHIBITION OF OVARIAN DEVELOPMENT IN THE SAKHALIN PINE LONGICORN, *MONOCHAMUS SALTUARIUS* (COLEOPTERA: CERAMBYCIDAE)

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The Sakhalin pine longicorn, *Monochamus saltuarius* Gebler (Coleoptera: Cerambycidae), is an insect vector of the pine wilt nematode (PWN), *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle, and is widely distributed in central Korea. *M. saltuarius* is a forest pest that seriously damages *Pinus densiflora* and *Pinus koraiensis* forests. We examined the effect of electron beam irradiation on the mating, DNA damage and ovarian development of *M. saltuarius* adults and sought to identify the optimal dose for sterilizing insects. When the adults were irradiated with electron beams, both females and males were completely sterile at 200 Gy. The number of eggs decreased with increasing doses. In a reciprocal crossing experiment between unirradiated and irradiated adults, the reproductive ability of wild adults was recovered by crossing with wild adults even after crossing with sterile adults. When a pair of unirradiated adults (♀- or ♂-) and 10 or 20 irradiated adults (♀+ or ♂+) were kept together, the control effect was as high as 80 ~ 90%. After electron beam irradiation at 200 Gy, the DNA of *M. saltuarius* adults was damaged, the ovarian development of female adults was inhibited, and the level of vitellogenin was significantly decreased compared with that in unirradiated female adults. These results suggest that pine wilt disease can be effectively controlled if a large number of sterilized *M. saltuarius* male adults are released into the field.

Keywords: Electron beam, *Monochamus saltuarius*, sterile insect technique, DNA damage, ovarian development

PO007

A COMPARATIVE ANALYSIS ON LIFE TABLE OF *NILAPARVATA LUGENS* BETWEEN RESISTANT GENES BPH1, BPH2, AND BPH18

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Brown planthopper (BPH), *Nilaparvata lugens* Stål (Hemiptera: Delphacidae), is one of the important migratory pests damaging to rice in Korea. It has been annually invaded from tropical and subtropical areas by continental air streams. It is necessary to determine the resistance levels to rice varieties for control efficiency. The development, survival, and reproduction of BPH populations (1980s, 2015_NH, 2016_HD, 2016_SC) was reared in the laboratory at 25±2°C with 65±5% RH and a photoperiod of 16L: 8D on five rice varieties of: Dongjin 1(none), Chungchungbyeo(Bph1), Jangseongbyeo(Bph1), Chinnongbyeo(Bph2) and Jungmo 1045(Bph18), respectively. The information obtained is reported in Meyer *et al.* (1986) presented the JackKnife method, we created the life table statistics respectively. BPH nymphs successfully survived on all rice varieties, although survival rate was low on Chungchungbyeo (12.0%) and Jangseongbyeo (26.0%) except 1980s-BPH. The developmental period of immature stages ranged from 11.5±0.61 d on Chungchungbyeo to 20.7±0.90 d on Jangseongbyeo and Jangseongbyeo were generally longer than Dongjin 1. The reproductive period and female longevity were longest on Dongjin 1, Chinnongbyeo and Jungmo 1045 while the highest fecundity of BPH being also observed on these three rice varieties. The highest and lowest net reproductive rates (R0) were detected on resistant rice varieties, Chinnongbyeo and Jangseongbyeo, respectively. Intrinsic rates (rm) of population increase show a similar level from Chinnongbyeo, Jungmo 1045 to Dongjin 1. The Chungchungbyeo and Jangseongbyeo showed lower than Dongjin 1. Mean generation time (T) was the longest on resistant rice varieties Jangseongbyeo. These population parameters showed that BPH can successfully survive and reproduce on Chinnongbyeo, and the resistance of Jangseongbyeo is the strongest.

Keywords: Brown planthopper, Life table, Resistant genes, Survival rate

PO008

FIRST YEAR OF SURVEY ON HETEROPTERANS ASSOCIATED TO THE INFLORESCENCE OF INDUSTRIAL HEMP (*CANNABIS SATIVA*) IN CENTRAL ITALY

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Hemp (*Cannabis sativa* L.) is one of the oldest cultivated crop which had a large diffusion principally for fiber and seed production. Since the 19th century in many countries, including Italy, the hemp cultivation was gradually interrupted principally due to the competition with cotton and synthetic fibers and also for the prohibition of cultivation of the congeneric specie, *C. indica*, used as a narcotic. Currently, despite a growing interest in hemp cultivation which can provide, among the various products, high quality oil contained in the seeds, there is a lack of knowledge on many aspects of its cultivation, including the detrimental action of insects which can preclude or reduce the yield. Therefore, we report the results of the first year of a survey conducted in a hemp crop for seed production (cv Ferimon) in central Italy (Viterbo) with the objective to evaluate the presence of insect pests on *C. sativa* inflorescences. The investigation was carried out in the summer of 2017 at the experimental farm of Tuscia University. The treatments applied on the hemp crop were: 2 levels of irrigation (irrigated and no irrigated), 3 levels of nitrogen fertilization (0-75-150 kg N ha-1), and 3 levels of phosphorus fertilization (0-45-90 kg P2O5 ha-1). During the survey we ascertained the presence of four Heteropteran species: *Nezara viridula* (L.), *Dolycoris baccarum* (L.), *Piezodorus lituratus* (F.) (Hemiptera: Pentatomidae) and *Liorhyssus hyalinus* (F.) (Hemiptera: Rhopalidae). *L. hyalinus* was by far the most present, in both irrigated and not irrigated treatments. However, our results did not highlight statistical differences in terms of heteropterans collected in fertilized plots both with nitrogen and phosphorus, while significant differences were observed between irrigated and not irrigated treatments.

Keywords: Cannabaceae, true bugs, Hemiptera, hemp seeds

PO009

MONITORING ACTIVITIES ON INVASIVE FRUIT FLIES (TEPHRITIDAE, DIPTERA) IN AUSTRIA

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Fruit flies (Tephritidae, Diptera) are a worldwide threat to their cultivated and wild hostplants, as up to 200 species are considered as pests infesting ripening fruits. Compared to countries with more favourable climatic conditions for the development of fruit flies the number of harmful species in Austria is low. Only the European cherry fruit fly (*Rhagoletis cerasi*) is considered as an important native pest fruit fly. However, high interception numbers of tephritids at the entry points (*e.g.* airports, harbours) of the European Union and previous findings of non-native fruit flies in Austria, emphasized the establishment of regular national monitoring activities for species of economic importance. These activities seem rather important since several host plants for some of the most destructive invasive tephritid species are cultivated in the warmer areas of the country. Therefore, these actions were intensified since 2016 focusing on alien species from the genera Ceratitis and Bactrocera. While in 2016 a total of 68 traps with male-specific lures were employed to attract and catch the adult flies on 17 sampling sites, in 2017 the number of sampling sites was extended to 27 with a total of 104 traps with male-specific lures and in addition 2 traps with lures for female fruit flies. Trapping activities started in June and traps were serviced in a two-week interval until October/November. Traps were placed on sampling sites with host plants, mainly peach, apricot but also other suitable fruit trees. Identification of caught flies was carried out on morphological basis and confirmed with molecular diagnostic methods (including barcoding) for single flies. Furthermore molecular sequencing was used to determine the potential source of the caught specimens. Monitoring activities resulted in catches of several hundred fruit flies from the genera Ceratitis and single individuals of the genera Bactrocera. Detailed results of the monitoring will be presented.

Keywords: Tephritidae, fruit flies, monitoring, Austria

PO010

SURVEY OF NEMATODES ASSOCIATED WITH PINEWOOD IN SOUTHERN ITALY AND LEBANON

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The pinewood nematode, *Bursaphelenchus xylophilus*, is the causal agent of the devastating "pine wilt disease" (PWD). It is a quarantine pest originating from North America, and a major invasive pine pest in Eurasia. In Italy and Lebanon, it has never been detected so far, even if its vector *Monochamus* sp. is present in both countries. To identify the nematofauna associated with the main pine species, surveys in Lebanon (Horsh Beirut and Maten Region) and Southern Italy (Ionic coast and Salenton subregion) have been conducted. Wood samples were taken from pine trees showing declining symptoms; in addition, an evaluation has been carried on the presence of PWN and other nematode species associated with bark and wood boring insects. Nematodes have been extracted from pinewood, and morphological and molecular identification will be performed to identify the species. Considering the wide distribution of *Monochamus* spp., conifers and climate conditions, Italy and Lebanon show favorable conditions for the development, establishment and spread of PWN. This reinforces the importance of the monitoring system set up which has to contribute to the early detection of the nematode or latent infections.

Keywords: Quarantine Nematodes, Pine, survey, South of Italy, Lebanon

PO011

LEPTOGLOSSUS OCCIDENTALIS (HEIDEMANN, 1910), AN INVASIVE SPECIES ATTACKING CONIFERS IN LEBANON: ASSESSMENT OF DAMAGE AND PRELIMINARY LABORATORY CONTROL BY THE *ENTOMOPATHOGEN BEAUVERIA BASSIANA*

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The western conifer seed bug, *Leptoglossus occidentalis* (Heidemann, 1910), is an alien invasive species originating in North America and attacking conifers in Lebanon. The first record of the pest in Lebanon was in 2015. It got established in different pine forest regions from North to South of Lebanon and was associated with dry cone syndromes. The objectives of the study were to assess the damage on pine nuts associated with the western conifer seed bug and to evaluate the efficacy of a biological control agent, *Beauveria bassiana*, on the immature stages of *L. occidentalis* under laboratory conditions. Assessment were conducted in 12 different villages to estimate the real impact of *L. occidentalis* on the cones. Empty kernels were examined for insect damages and results indicated that more than 90% of the empty kernels were associated with feeding damage caused by *L. occidentalis*. Chemical control of this pest is problematic because of the ecology of the insect and the restriction of chemical pesticides uses in forest ecosystems. The entomopathogenic fungus *Beauveria bassiana*, a potential microbial control agent against immature stages of *L. occidentalis* was evaluated under laboratory conditions. Two concentrations of conidial suspension were applied topically on eggs and first nymphal instars: 50 conidia/immature and 500 conidia/immature. The hatching of eggs treated with conidial suspension was 40% lower compared to 100% in the control. *Beauveria bassiana* caused 100% mortality of first instar and sporulation of the insects' cadavers occurred after 3 weeks. The present study shows that *B. bassiana* is a potential biocontrol agent to reduce the population of the alien insect.

Keywords: *Leptoglossus occidentalis*, pine, *Beauveria bassiana*, entomopathogen, alien invasive species

PO012

A FIRST WORLDWIDE MONITORING OF INVASIVE MEDITERRANEAN PINE BARK BEETLES (COLEOPTERA: CURCULIONIDAE, SCOLYTINAE)

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In recent decades some Mediterranean species of pine bark beetles (Coleoptera: Curculionidae, Scolytinae) became established in temperate countries of the southern hemisphere, posing a novel threat to large pine plantations. Our objectives were to investigate i) the presence of Mediterranean pine bark beetles (Orthotomicus, Hylurgus, and Hylastes) in countries of the southern hemisphere; ii) the main biological features of the species in the invaded areas; iii) the trapping performance of different blends of generic lures. In 2017, a network of pheromone traps was installed in 6 newly invaded areas of USA and the southern hemisphere (Australia, New Zealand, South Africa, Argentina, Uruguay), and for comparison in 6 native European countries

(Italy, Portugal, Spain, France, Greece, Hungary). Half of the traps were baited with α-pinene and ethanol, and the other half with α-pinene, ethanol, and bark beetle pheromones (Ipsdienol, Ipsenol, and Z-verbenol). Traps were emptied every second week. Five Mediterranean species (*Hylurgus ligniperda*, *Hylastes ater*, *H. angustatus*, *Orthotomicus erosus*, and *O. laricis*) were found in non-European countries. Specifically, H. ligniperda and H. ater were the species invading most of monitored countries (5 and 3 countries, respectively), while *O. laricis* and *H. angustatus* occurred only in one country (Argentina and South Africa, respectively). Populations occurring in the invaded areas show phenology and voltinism similar to the populations occurring in the native areas (Europe). Despite the large variations among species and countries, most species were trapped mainly with the blend containing bark beetle pheromones, except *O. erosus* that was more attracted by α-pinene and ethanol alone. These results pose the first step towards the development of an international monitoring protocol based on multi-lure traps for the interception of invasive species.

Keywords: Biological invasions, pine pests, international survey

PO013

DROSOPHILA SUZUKII FLIGHT ACTIVITY AND FEMALES REPRODUCTIVE STATUS IN AN OVERWINTERING SITE OVER A FOUR-YEAR PERIOD

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The invasive pest *Drosophila suzukii* (Matsumura) was first reported in South Tyrol in 2010 and it has rapidly established in the entire province. During winter, the fly can survive in various sites, where it was detected in monitoring traps. *D. suzukii* was found in high numbers especially in a wooded area, where the pest is present since 2012. This site is characterized by the presence of several evergreen plant species such as ivy (*Hedera helix* L.) and butcher's broom (*Ruscus aculeatus* L.), which could possibly provide shelter to *D. suzukii* during the winter months by creating a milder microclimate under the foliage. Moreover, these plants represent potential non-crop hosts for the pest in the early season. Therefore, the aim of the study was to investigate which conditions favor *D. suzukii* overwintering and its reproductive physiology. *D. suzukii* abundance was recorded weekly with baited traps over a four-year period. Trap captures of the coldest months (October to March), air temperature and precipitation were analyzed to understand the influence of climatic conditions on the pest's abundance. The reproductive status of females was assessed during the late dormant period to investigate the environmental conditions in which oogenesis starts. Ovarian maturation was evaluated by dissecting the insects and distinguishing five developmental stages of the eggs starting from March. The percentage of mature eggs at different dates was correlated with the accumulated degree days (DD) in the 4 subsequent years. The preliminary results of this study may contribute to understand the overwintering capability of *D. suzukii* populations.

Keywords: Climatic conditions, ovarian development, early spring

PO014

OLIVE CULTIVATION AND THE MAIN STRATEGIES TO CONTROL OLIVE KEY PESTS IN IRAQ

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The olive (*Olea europaea* L.) is among the oldest known cultivated trees in the world. It has been cultivated since the beginning of historical times in its native Asia Minor and spread very early to all the Mediterranean countries and later in many other areas of the American continent suited to its cultivation. Although Iraq is now a minor producer of olives, the olive cultivation is beginning to grow and prosper recording a production of 24.768 tonnes in 2014 using local cultivar as Pa'shiqi, Ashrassi and Dahkan and European cultivar. The country conditions in the previous decades such as wars and crises, were led to neglect of scientific research and pest control methods in the agricultural sector. Unfortunately, all of those conditions led to introduce and spread the pests in the olive cultivated areas and has driven for adoption of fast control methods as a major way to control these pests. We will discuss the key pests of olive such as Olive –leaf midge, *Dasineura oleae* (Low, 1885), Olive scale, *Parlatoria oleae* Colvée, 1880, Olive Psyllid, *Euphyllura olivina* (Costa, 1839), Olive Eriophyid Mite, *Aceria oleae* (Nalepa 1900), and Olive Bark Beetle, *Phloeotribus scarabaeoides* (Bernard, 1788), which represent the most damaged pests on olive in Iraq, where losses attributed to them have reached 40% to 60% of annual yield of olive production. Also we will discuss the most common strategies to control those pests in Iraq, and keep them under threshold level.

Keywords: Middle East, Olive Iraqian varieties, Olive distribution, phytophagous, IPM.

PO015

ACLEES SP. CF. *FOVEATUS* VOSS, THE NEW THREAT FOR *FICUS CARICA*: LABORATORY AND FIELD CONTROL TRIALS

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Invasive alien species represent a major disruption for all biotic systems, managed and wild. Invaders can have economic and human health impacts as well as degrading many system properties, including biodiversity. In Italian environment, fig crops are relatively pest- and disease-free, but plants are sometimes attacked by a variety of borers, mealybugs, and scale insects. No conventional insecticides are currently labeled for control. For these reasons, in many of our environments the fig crop is seen as related to the natural environment free of chemicals. Recently a new and exotic pest belonging to the genus *Aclees* (Coleoptera: Curculionidae), was reported as responsible of severe damage in many Italian fig nurseries and orchards, particularly in the Central Northern part (Tuscany, Liguria and Latium region). The Curculionidae belonging to Molytinae family, tribe Hylobiini, is reported as *Aclees* sp. cf. *foveatus* Voss but since the taxonomy of the genus is under review, the specific attribution is to be validated. The information on the biology and ethology of the species in Italian environments as well as for the control methods, are very scanty. Apparently were not at the time find fig cultivars resistant to the attacks of the pest, or control strategies capable of containing the infestations of this beetle; moreover the presence of the insect from nurseries to the scattered figs, is to assume that if not addressed systematically this problem, the fig cultivation on the territory could be decimated. Laboratory trials were setup:substances with biocidal and/or repellent activity were tested to check their efficacy against adults stages of the weevil *Aclees* sp. At the same time in fig crops of Central Italy, where *Aclees* sp. populations have already spread, different control strategies that are effective and at the same time safe from a toxicological and environmental point of view, were studied.

Keywords: Fig tree, curculionid, adult control, modified clays, *Beauveria bassiana*

PO016

NATIVE PARASITOIDS ASSOCIATED WITH *DRYOCOSMUS KURIPHILUS* IN NORTHWESTERN SPAIN: BIOLOGICAL COMMUNITIES WITHIN GALLS OF AN ALIEN AND RECENT INTRODUCED CYNIPID PEST

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Dryocosmus kuriphilus Yasumatsu, 1951 (Hymenoptera: Cynipidae) also known as Asian chestnut gall wasp (ACGW) is a globally invasive species which has spread from China to Japan, USA and more recently Europe, that causes severe damages in chestnut trees (*Castanea* spp. In Galicia (northwestern Spain) ACGW was firstly detected in 2014, being currently spread throughout this region, which is one of the largest chestnut production areas of Spain and Europe. Description of the new recruited parasitoids by ACGW, mainly from the native oak cynipid gall wasp parasitoid communities is useful to complement the monitoring and control management of the classical biological control using *Torymus sinensis* (Hymenoptera: Torymidae), and for the identification of possible alternative biological control agents in the native recruited community. A study of the Chalcidoidea parasitoid community recruited by ACGW in Galicia was carried out over one-year period, during spring and summer of 2017. An approximate number of 15.000 galls were collected on circa 100 sites throughout the Galician territory and more than 1100 parasitoids emerged. Collected galls were arranged in emergence cages, and all the emerged Chalcidoidea and Cynipidae species were identified. Sixteen native parasitoid species belonging to seven genera of six Chalcidoidea families were identified, of which seven species were new records for the parasitoid community associated with ACGW in Galicia: *Bootanomyia dorsalis* (Fabricius), *Bootanomyia* sp. nr dorsalis, *Eurytoma setigera* Mayr, *Sycophila flavicollis* (Walker), *Mesopolobus fasciiventris* Westwood, *Mesopolobus lichtensteini* (Mayr) and *Mesopolobus sericeus* (Förster). Dominant species were *Eupelmus urozonus*, *Torymus flavipes* and *Eurytoma brunniventris* while the presence of three species was recorded occasionally. For the first time in Europe and Spain, one inquiline cynipid species, *Synergus facialis* Hartig (Hymenoptera, Cynipidae) was found in the recruited food web of ACGW. An analysis on differences among parasitoid communities on ACGW related to climatic areas and composition of native Quercus species adjacent to the sampled sites is included.

Keywords: *Dryocosmus kuriphilus*, invasive species, chestnut, biological community, Iberian Peninsula

PO017

GENETIC CHARACTERIZATION OF *CERATITIS CAPITATA* WIEDEMANN. (DIPTERA: TEPHRITIDAE) POPULATIONS SAMPLED FROM CITRUS GROWING REGIONS OF TURKEY BY ISSR MARKERS

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Ceratitis capitata Wiedemann. (Diptera: Tephritidae), the Mediterranean fruit fly (medfly), is the most dominant and important pest on fruit orchards in the world, including tropical and sub-tropical areas. By damaging various agricultural products, it causes huge amount of economic losses annually. This polyphagous species originated from Africa and it has spread from Africa to the Mediterranean basin. Inter-simple sequence repeats (ISSRs) are regions in the genome flanked by microsatellite sequences. PCR amplification of these regions using a single primer yields multiple amplification products that can be used as a dominant multilocus marker system for the study of genetic variation in various organisms. The purposes of this study was the genetic characterization of *Ceratitis capitate* populations sampled from important citrus growing regions of Turkey by using ISSR markers. For this purpose, adult medfly samples were collected from Adana, Mersin, Antalya, Muğla, Aydın, İzmir and Yalova populations. DNA isolation was performed by using Lifton method. Fifteen individuals from each population were screened with six ISSR primers. Data analysis was performed by using POPGENE version 1.32 and GenAlEx version 6.5 software programs. The mean values for observed number of alleles (Na) was 1.98±0.13, effective number of alleles (Ne) was 1.33±0.29 and Nei's gene diversity (h) was 0.22±0.15. AMOVA results revealed that only a limited degree of total variation was attributed to among populations, while the remaining high level of variation was due to within population variation. Findings of this study provided important information about the population structures of Turkish medfly populations and is helpful to contribute to the development of agricultural pest management strategies.

Keywords: Mediterranean fruit fly, *Ceratitis capitata*, Genetic variation, ISSR markers

PO018

CHEMICAL ECOLOGY OF THE BRONZE BUG, *THAUMASTOCORIS PEREGRINUS*: BASIC AND APPLIED QUESTIONS FOR STUDYING A FORESTRY PEST

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Forest plantations have expanded greatly in southern South America, mostly based on Eucalyptus species for pulp production. The bronze bug, *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae), is a relatively new invasive eucalypt pest in the region, also present in Africa, southern Europe, Israel and New Zealand. No damage estimates and no defined control measures are currently available for this pest. Semiochemicals are promissory tools for the management of *T. peregrinus* through attractants and/or repellents that act as natural signals or cues for the insect. We have described a male-emitted pheromone, 3-methyl-2-butenyl butyrate, that attracts males, both juveniles and adults, but not females. This compound, with yet undefined ecological function, is emitted with a diel pattern by virgin males, a pattern that is suppressed by the presence of females. Since this species occurs in aggregations, our current hypothesis is that this compound is not an attractant pheromone, but rather a mediator of male-male interactions, possibly determining or correlating with dominance status. Using behavioral studies and individual analyses, we showed that 3-methyl-2-butenyl butyrate is present in higher amounts in males that perform as dominant in behavioral interactions. Hence, the compound appears to either determine male-male competition outcomes, or correlate with other characters involved in such competition. We also show that the amount of 3-methyl-2-butenyl butyrate does not correlate with body weight, hence it is not a byproduct of male size, and that males in isolation produce similar amounts than dominant males, suggesting that submissive males decrease this potential signal for male dominant status. We have also found that the presence of synthetic 3-methyl-2-butenyl butyrate in cages with males and females slightly but significantly decrease the number of eggs laid by the females, suggesting that the compound may have potential as a mating disruptant for the bronze bug in eucalypt crops.

Keywords: Pheromones, *Thaumastocoris peregrinus*, eucalypt pest

PO019

POPULATION DYNAMICS OF *DROSOPHILA SUZUKII* AND FIRST DATA ON ITS PARASITOIDS ON MOUNT ETNA (ITALY)

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Drosophila suzukii (Diptera: Drosophilidae) is a serious pest of various fruit crops cultivatedon Mount Etna (Sicily, Italy). Population dynamics of *D. suzukii* have been studied for one year exposing traps baited with Droskidrink in five locations of two major host crop production districts of Mount Etna. Traps were exposed in three cherry orchards located in the East side (530-810m a.s.l.), and in two strawberry farms located in the North-West (880-1070m a.s.l.) of Mount Etna. The East locations were warmer than the North-West ones, and flies were captured all year around, except in August. While, in the North-West locations there were no fly captures during the winter. There were two *D. suzukii* capture peaks in all locations, one in late spring and the second in autumn. The *D. suzukii* sex-ratio had a variable trend along the seasons, with more females being caught in late winter, early spring and in the summer. Two occasional samplings of parasitoids were conducted by exposing sentinel fruit traps baited with *D. suzukii* young instars. The sampled species were the larval parasitoid *Leptopilina boulardi* (Hymenoptera: Figitidae), and the pupal parasitoids *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae) and *Trichopria drosophilae* (Hymenoptera: Diapriidae). All species were tested in the laboratory for their ability to parasitize *D. suzukii*, but only the pupal parasitoids were able to successfully attack this host. While, *D. suzukii* larvae resisted to the *L. boulardi* parasitization by encapsulating parasitoid juveniles. Altogether, these findings provide new knowledge of the status of this pest and its fortuitous parasitoids in a particular southern Mediterranean context, characterized by soft-skinned fruit farms surrounded by large areas of natural landscapes with an abundant presence of the pest wild hosts.

Keywords: Biocontrol, cherry, invasive species, Spotted wing drosophila, strawberry

PO020

HARMONIZING AND UPDATING ALIEN INSECT DATASETS: THE INTERREGIONAL APPROACH OF THE ALIEM PROJECT

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Invasive alien species know no borders and collaborations among different administrative units is crucial at different levels, both within the same country and between countries. The invasion of the yellow-legged hornet, *Vespa velutina*, is a representative case, highlighting that we cannot remain within our own borders to face common interregional issues. Indeed, the purpose of the ALIEM project (Action pour Limiter les risques de diffusion des espèces Introduites Envahissantes en Méditerranée; 2014 - 2020 INTERREG V-A Italy – France, Maritime) is to develop common best practices, share methods and join efforts to tackle biological invasion and their impacts, especially on biodiversity. Distribution and other features regarding alien insect species, such as native and invaded range, pathways of introduction, invasivity, will be updated for each of the administrative units involved (*i.e.* Liguria, Tuscany, Sardinia in Italy; Corsica, Var and Alpes-Maritimes in France). Lists and information from already existing databases, publications, past and ongoing projects will be used and updated. Moreover, beside reviewing and including new information regarding the species' impact, we will focus for the first time on those species which exert their impact onto natural protected areas of the Natura 2000 network, for the five regional territories involved. An online interactive platform reporting harmonized regional alien insect datasets from different units is an expected deliverable. Once built, the framework of common elements will ensure the coherence of this output and provide a positive impact on the whole ALIEM area. An updated report of the work done by the ALIEM entomological group is reported here to discuss the issues and opportunities related to sharing and harmonizing datasets from different research/management and administrative units, with a focus on ecological impact issues.

Keywords: Alien species, alien insects, database, impacts, natura 2000

PO021

Poster withdrawn.

PO022

FUNCTIONAL AND GENETIC CHARACTERISTICS OF CHLORANTRANILIPROLE RESISTANCE IN THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (LEPIDOPTERA: PLUTELLIDAE)

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The diamondback moth (*Plutella xylostella*) is a globally distributed and important economic pest, and it has developed resistance to all conventional insecticide classes used in the field. Chlorantraniliprole is a new chemical class of insecticide that acts as a conformation-sensitive activator of the insect ryanodine receptor (RyR). In the present study, a field strain (16.3-fold resistance to chlorantraniliprole) was collected in Korea and lab-selected with chlorantraniliprole for more than one year. The resulting strain presented 2,157-fold resistance to chlorantraniliprole. A point mutation (G4946E) in the RyR gene was observed at a high frequency in the resistant strain. Enzyme assays indicated that glutathione S-transferase (GST) and P450 activity in the resistant strain were 2.4- and 1.96-times higher than that of the susceptible strain, respectively. The expression of the RyR, GST (sigma, omega, and zeta) and *CYP321E1* gene was higher in the resistant strain than in the susceptible strain. The F1 progeny resulting from reciprocal crosses did not reveal maternal effects or a diamide-susceptible phenotype, which suggests an autosomal nearly recessive mode of inheritance. In addition, we surveyed the susceptibility to 13 insecticides (3 diamides, 2 synthetic pyrethroids, 2 spinosyns, 1 organophosphate, 1 oxadiazine, 1 avermectin, and 3 others) in the chlorantraniliprole-resistant strain. The resistant strain exhibited high cross-resistance to flubendiamide (5,910 fold) and showed no cross-resistance to spinetoram, spinosad, indoxacarb, and metaflumizone. These results can serve as an important basis for guiding the use of insecticides in the field.

Keywords: Chlorantraniliprole, *Plutella xylostella*, resistance mechanism, ryanodine receptor

PO023

MODELLING THE REPRODUCTION STRATEGY OF *RHOPALOSIPHUM PADI* (HEMIPTERA: APHIDIDAE)

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Longevity, survivorship, and fecundity of *Rhopalosiphum padi* (L.) were studied on barley (*Hordeum vulgare* L.) at 10, 15, 19, 22, 26 and 31˚C (all ±0.5), 60±5% RH and 16:8 h L: D for developing a model of reproduction.The preliminary results revealed the impact of temperature on the mentioned features. Longevity decreased as temperature leveled up from 10 to 31ºC and ranged from 43.9±5.9 to 15.56±1.13 days. Similarly, the highest and lowest fecundity was observed at 25˚C and 10˚C (52.94±5.24 and 20.95±2.54 nymphs. female-1), respectively. Moreover, the developmental rate (1/median longevity) used to calculate physiological age of adult aphids was well described by modified model of Sharp-DeMichele in the range of the studied temperatures. Cumulative age- specific nymph production and survival of adults were fitted well to the two-parameter Weibull equation and fitted well to a reverse logistic curve, respectively. These three temperature-dependent traits (age-specific cumulative nymph production, temperature-dependent total fecundity, and age-specific survival rate) were integrated into a model of reproduction. Simulation of reproduction strategy using the model has the potential to be effective for predicting patterns of occurrence of *R. padi* nymph populations on barley fields which may contribute to a better understanding of reproduction strategy and behavior of *R. padi*.

Keywords: Reproduction strategy, Physiological age, Survival, Temperature, *Rhopalosiphum padi*

PO024

TWO NEW POINT MUTATIONS AT CYTOCHROME B OF ACEQUINOCYL RESISTANT TWO-SPOTTED SPIDER MITE, *TETRANYCHUS URTICAE*

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Two-spotted spider mite, *Tetranychus urticae* was collected from the rose greenhouse in Gimhae, Gyeongnam Province in January 2001. This population has been selected for sixteen years with acequinocyl, and increased 4,237 fold in resistance as compared with susceptible strain (S). Cross resistance of acequinocyl resistant (AR) strain to eleven miticides was investigated. The AR adults strain exhibited high cross resistance to cyflumetofen (117 fold), etoxazole (322 fold), pyridaben (2,439 fold), and spiromesifen (510 fold). The detoxifying enzymes of the AR strain showed 1.8 ~ 2.3 fold activity in GST as compared with S strain. The expression of the GST (delta 1) gene was higher in the resistant strain than in the S strain. The F1 progeny resulting from reciprocal crosses was shown to be maternally inherited. By comparing the mitochondrial cytochrome b (cytb) sequence, two-point mutations were detected at a high frequency in the AR strain. These results increase the understanding of the mechanisms of acequinocyl miticide resistance in *T. urticae*.

Keywords: *Tetranychus urticae*, Acequinocyl, Cross resistance, Point mutation, Cytochrome b

PO025

STRESS RESISTANCE AND IMMUNE SYSTEM IN LADYBIRDS: IS THERE A DIFFERENCE BETWEEN NATIVE AND INVASIVE SPECIES?

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The harlequin ladybird, *Harmonia axyridis*, is considered to be one of the most successful invasive insect species. It has been hypothesized that successful invasive species could systematically differ from other species by their specific species traits. Resistance to environmental stress and against various pathogens are supposed to be among the most important traits related to invasive success. In our study, resistance against starvation and desiccation, and immune system function of *Harmonia axyridis* was compared with several other ladybird species native to Central Europe. Longevity of invasive *Harmonia axyridis* adults under stressful conditions (no food and no water) was much shorter than longevity of *Coccinella septempunctata*, representing native ladybird species of similar size. In general, longevity of particular ladybird species was related to species body size and habitat preferences: larger species outcompeted smaller in their stress resistance and species from dry habitats outcompeted these from mesic habitats. Longevity of *Harmonia axyridis* was comparable to longevity of several smaller native ladybird species. Immune system of invasive *Harmonia axyridis* clearly outcompeted immune system of *Coccinella septempunctata* in means of antimicrobial activity against *Escherichia coli*, but there was only a small difference in immune function between *Harmonia axyridis* and *Ceratomegilla undecimpunctata*. The two later species are closely related to each other and thus actual functioning of immune system could be a result of species evolutionary history. However, future research focused on immune system function in additional ladybird species is needed to disentangle sources of interspecific variability.

Keywords: Antimicrobial activity, desiccation, *Harmonia axyridis*, invasive species, starvation

PO026

EXPANSION OF BROWN MARMORATED STINK BUG (*HALYOMORPHA HALYS*) IN SERBIA

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Invasive species, native to East Asia, Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys* (Stål, 1855) (Heteroptera, Pentatomidae) is becoming cosmopolitan species inhabiting USA and Europe as well. Since its first records in Europe, dating back in 2004 in Liechtenstein, *H. halys* in relatively short time invaded many countries. After the first records in neighboring Hungary (2013) and Romania (2015), the first BMSB specimens were photographed in Serbia, in small town Vršac, at Serbian - Romanian border, in October 2015. Several days later, specimens were also recorded in the urban zones of Belgrade, the capitol. Surveys conducted in following two years showed that *H. halys* successfully survived first winters and started to establish in newly invaded area. Increase of population was observed, together with spread through the country. By the end of two vegetative seasons (2016 and 2017) beside urban zones and findings on platanus trees and hibiscus, the hosts list was expanded by two important agricultural crops, tomato and soybean. So far, agricultural producers didn't face economic losses in tomato and soybean production due to low abundance of BMSB specimens, but monitoring and surveys were planned in the coming years, with expanded list of potential hosts. Beside its highly polyphagous preferences and huge pest potential, *H. halys* is also recognized as serious nuisance species. Most of the first findings of BMSB in Serbia were in and around man made facilities, and species caused nuisance problem and panic due to high population density in urban zones, in autumn 2016 in Belgrade. Evident establishment of *H. halys* in urban and agricultural areas in Serbia, and its spreading over the country put this species in focus in braoden list of areas and hosts. This work presents expansion of *H. halys* in three years and observation of its potential threat to agricultural areas in Serbia.

Keywords: *Halyomorpha halys*, establishment, soybean, Serbia

PO027

CHARACTERIZATION OF STERILITY MECHANISM IN *SPODOPTERA LITURA* BY IONIZING RADIATION

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The armyworm, *Spodoptera litura* (F.) is a polyphagous and important agricultural pest worldwide. This study investigated the effects of electron beam irradiation on the development and reproduction of *S. litura*. When eggs were irradiated with 100 Gy, egg hatching was completely inhibited. When the larvae were irradiated, the larval period was significantly delayed, depending on the doses applied. At 150 Gy, the fecundity of adults that developed from irradiated pupae was entirely inhibited. However, electron beam irradiation did not induce the instantaneous death of *S. litura* adults. When the pupae were irradiated with electron beams, the ovarian development of newly emerged adults was suppressed. However, there was no significant difference when the adults were irradiated. The expression level of vitellogenin (Vg) and Vg receptor (VgR) gene was significantly decreased at over 100 Gy. Electron beam irradiation also reduced feeding, including total protein level and storage protein on *S. litura* larvae. The expression of abnormal wing disc (AWD) and trehalose-6-phosphate synthase genes were lower in irradiated larvae than in nonirradiated larvae. This result suggests that electron beam inhibited the ovarian development and then thus cause infertility.

Keywords: *Spodoptera litura*, ionizing radiation, sterility

PO028

POSITIVE INTERACTIONS BETWEEN TWO PINE BORERS: *SIREX NOCTILIO* AND *TOMICUS PINIPERDA*

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At low population densities there is a tendency for *Sirex noctilio* (*Hymenoptera Siricidae*) to occur within pine stands also being attacked by *Tomicus piniperda* (Coleoptera, Scolytinae). A regional survey of 1.015 dying pines indicated that *S. noctilio* and *T. piniperda* were about twice as likely to occur in the same tree as expected by chance. In the summer of 2014, we marked and mapped 100 intermixed pine trees (30 codominant and 70 intermediate) within ~ 1 ha of *Pinus pinaster* forest along the growing edge of an infestation of *T. piniperda*. We measured resin defences of each tree previous to the seasons of attack by *S. noctilio* and *T. piniperda*, and three times more — before and after *T. piniperda* flight and finally in the following spring. We scored each tree for attacks by both species during 3 years. Additionally we inoculated 10 live trees with 15 pairs of *T. piniperda*, 5 of them previously attacked by *S. noctilio*. Intermediate trees had significantly lower resin flow than codominant trees. By summer of 2017 *S. noctilio* had attacked 57 of our study trees, being concentrated on intermediate trees with low resin defences. *T. piniperda* had attacked a total of 53 study trees, also strongly biased toward intermediate trees with low resin defences; 44 of the 53 had been previously attacked by *S. noctilio*. A total 28 study trees died, all of them were trees that had low resin defences in fall and were subsequently attacked by both species. The length of *T. piniperda* galleries and the number of eggs laid was significantly higher on trees previously attacked by *S. noctilio*. At least in Galicia, tree death is essential for successful reproduction by *S. noctilio*, so both species seem to benefit each other at low population rates.

Keywords: Abundance, invasive pest, pine, woodwasp, Pine shoot beetle

PO029

TESTING NEW TRAPPING PROTOCOLS FOR THE MONITORING OF NATIVE AND ALIEN LONGHORN BEETLES (COLEOPTERA: CERAMBYCIDAE)

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Introduction of alien wood boring beetles within wood materials associated with international trade is a problem of global relevance. The aim of this study was to identify the most effective early-detection protocol for trapping native and alien longhorn beetles. Specifically, we tested three variables known to potentially affect beetle trapping: trap colour (green or purple), trap height (low, in the understory, or high, in the canopy) and the type of

attractive lure (ethanol or a multi-lure combination of ethanol plus pheromones specific for longhorn beetles). The study was conducted at eight sites located in Venice and Treviso provinces (NE Italy): at each site, eight multi-funnel traps were set up following a factorial experimental design for testing all possible combinations among the tested variables. We found that species richness was affected both by the type of attractive lure (the multi-lure was more effective than ethanol) and the combination of lure and trap height (traps baited with the multi-lure and placed in the canopy were significantly more effective than those located in the understory). Catches of two most abundant exotic (*Xylotrechus stebbingi*, *Neoclytus acuminatus*) and native species (*Plagionotus detritus*, *Xylotrechus antilope*), were greater in traps baited with the multi-lure than with ethanol. Traps in the canopy caught more *N. acuminatus* than traps in the understory. There was a significant interaction between lure type and trap height for catches of *P. detritus*, with greatest catch in traps baited with the multi-lure and placed in the canopy. This information will be useful for monitoring programs carried out in Italy and abroad in port areas and their surrounding woodlands.

Keywords: Survey, biological invasions, wood-boring beetles

PO030

GENETIC VARIABILITY OF *HALYOMORPHA HALYS* (STÅL) (HEMIPTERA: PENTATOMIDAE) AND ITS SYMBIOTIC BACTERIA '*CANDIDATUS PANTOEA CARBEKII*' ACROSS NATIVE AND NEWLY COLONIZED REGIONS

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The increase and development of the world trade system, during the last century, have facilitated the biological invasions of several exotic pests in new areas with important environmental and economic consequences for agriculture. The brown marmorated stink bug, *Halyomorpha halys*, is an important pest of agricultural crops in invaded regions. Native to southeastern Asia, in the last decades *H. halys* has invaded several countries of Asia, America and Europe causing considerable economic losses. In Europe, this exotic pest was first reported in Switzerland in the early 2000s. Since then, it has rapidly spread and established in several countries across this continent. Symbionts have been used to trace the origin and infer the routes of expansion of their hosts populations. *H. halys* harbors a primary, obligately-associated endosymbiont named '*Candidatus Pantoea carbekii*' with two different haplotypes retrieved in some populations sampled across the world. Despite the wide expansion range of *H. halys*, few European and native populations have been used to study the genetic variability of its symbiont. In the present study, the genetic diversity of both Ca. *P. carbekii* and their host was analyzed in different *H. halys* populations from native and introduced regions. New haplotypes from both the symbiotic bacteria and the host were retrieved in Europe, suggesting different invasion events and confirming, as proposed by other authors, that European populations are the result of multiple introductions, which are still in progress. The information obtained here combines data of the genetic variability of both *H. halys* and its symbiont and can contribute to elucidate some aspects concerning the pathways of expansion of *H. halys* in the European continent.

Keywords: Brown marmorated stink bug, symbiotic bacteria, invasive species, haplotype

PO031

SPREAD AND POPULATION GENETICS OF BIOLOGICAL CONTROL AGENT *TORYMUS SINENSIS* IN CROATIA, SLOVENIA AND HUNGARY

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Torymus sinensis (Hymenoptera: Torymidae), a classical biocontrol agent of chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), was released in Croatia, Slovenia and Hungary in 2015. Following the introduction, the research of parasitism rates and population genetic indices on 40 different sites was performed in order to monitor and evaluate the success of establishment. *T. sinensis* established viable and genetically diverse populations and successfully spread naturally from Italy across Slovenia to Croatia and Hungary. *T. sinensis* populations from release and non-release sites share common gene pool, adding to the hypothesis that the spread occurred naturally from Italy, even before official releases in Slovenia, Croatia and Hungary were done. Population genetic indices showed that populations of *T. sinensis* did not suffer from bottleneck-induced founder effect phenomenon. This rapid dispersal of *T. sinensis* was aided by high concentration of the host, as well as the lack of competition of native parasitoids.

Keywords: Classical biological control, parasitoid, haplotypes, dispersal, *Dryocosmus kuriphilus*

PO032

BIOLOGICAL INVASION BY *DACTYLOPIUS OPUNTIAE* (COCKERELL) (HEMIPTERA DACTYLOPIIDAE) IN THE MEDITERRANEAN BASIN

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Dactylopius opuntiae (Cockerell) belongs to the genus *Dactylopius Costa* that comprises 11 species characterized by some morphological structures (truncate dorsal setae and clusters of quinquelocular pores associated with tubular ducts) and by the presence of carminic acid that is extracted from the females to produce a natural red dye (*i.e.* carmine). The current diffusion in the world of the cochineals and their host plants (Cactaceae), both native to the Americas, is linked to the commercial trades that began with the Spanish conquistadores since 1493. From Spain, cactaceae spread to new areas and are now present worldwide, in some cases becoming weeds. *Dactylopius opuntiae* is noxious to the host plants that weaken and possibly die as a consequence of its trophic activity, so that it has been introduced, along with other biological control agents, in many parts of the world, in order to control cactus weeds. Recently, considering that in some areas, the role of the prickly pear [*Opuntia ficus-indica* (L.) Mill], changed (*e.g.* as in South Africa) because the plant is nowadays cultivated for human and animal consumption, the public's perception regarding this plant and its biological control agents (*e.g. D. opuntiae* and *Cactoblastis cactorum* Berg) is changed and the cochineal is currently perceived as being pests. Also in the Mediterranean basin, the cultivation of prickly pears as a crop is growing; in fact, the changing weather conditions and rising temperatures have a direct impact on the environment, facilitating the development of the plant as well as the survival of the cochineal. In this scenario, *D. opuntiae* has become a pest of prickly pear crops in Spain, France, Morocco, Lebanon and Israel, with heavy damages and socio-economic consequences. Other countries where the prickly pear is cultivated as in Sicily, are then considered as at extremely high risk of infestation.

Keywords: *Opuntia cochineal scale*, *Opuntia ficus-indica*, prickly pear pest

PO033

ENTOMOPATHOGENIC FUNGI AS A PREVENTATIVE BIOCONTROL MEAN OF DATE PALM COLONIZATION BY THE RED PALM WEEVIL

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The red palm weevil (RPW, *Rhynchophorus ferrugineus* (Olivier) first appeared in the Middle East in the 1980s and since has heavily damaged date production by destroying thousands of date palms. RPW infestation was first reported in Israel in the southern Jordan valley in 1999. Entomopathogenic fungi of the genus *Metarhizium* and *Beauveria* spp. are occasionally found to naturally infect RPW populations in Israel. Therefore, laboratory experiments were conducted to assess directive virulence of both *M. anisopliae* and *B. bassiana* isolates from the ARO collection of EPF. In laboratory screening, several *M. anisopliae* and *B. bassiana* isolates were tested toward *R. ferrugineus* larvae. The isolates were virulent toward the eggs, larvae and adult. Following these promising results we hypothesized that EPF could reduce or even prevent date palm colonization by newly hatched larvae. Both laboratory and palms experiments demonstrated the effectiveness of early treatment of surface with conidia as a preventative mean. The promising results indicate of an immense potential in developing and applying entomopathogenic fungi as a preventative mean, however research is still on going, focusing on formulation and delivery methods, which will be examined in date palm plantations.

Keywords: Biocontrol, entomopathogenic fungi, red palm weevil

PO034

MODELLING WESTERN CORN ROOTWORM WING STRUCTURE AND WING SHAPE DIFFERENCES BASED ON CROP RESISTANCE USING FINITE ELEMENT ANALYSIS

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Intercontinental introductions of the worst insect pest of corn, the western corn rootworm (*Diabrotica virgifera virgifera* LeConte), from North America into Europe are still occurring. Through such introductions the spread of resistant alleles to various control techniques can occur and render once effective control programs ineffective, and as such innovative pest monitoring and surveillance tools and technologies must be implemented to mitigate this. Previous studies have repeatedly demonstrated evidence of distinct corn rootworm wing shape changes related to resistance development. Here, we investigate corn rootworm intraspecific flight morphology, in addition to modelling wing structure and wing shape differences based on crop resistance using finite element analysis. A deeper understanding of rootworm wing shape and flight morphology, aspect ratio and flight efficiencies will assist with discerning which resistant phenotypes are most likely to invade geographic areas where they not yet present (ie. rotation resistant beetles entering Europe where such variants are absent). Such information is crucial to biosecurity measures and integrated pest management strategies for the western corn rootworm globally.

Keywords: Western corn rootworm, wing morphology, finite element method, invasive species, biosecurity

PO035

IMPACT OF THE BROWN MARMORATED STINK BUG, *HALYOMORPHA HALYS* (HEMIPTERA: PENTATOMIDAE) ON CHERRY

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The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is a highly polyphagous pest insect that feeds on more than 170 known host plant species. It is a non-native invasive species to both North America and Europe. The spread of this highly adaptable insect is threatening many different crops in newly infested areas, including cherry (*Prunus avium*). The following study took place in the Veneto region of Italy during the summer of 2017. Insect proof mesh cages containing varying densities of BMSB were placed around clusters of 10 cherries for 7-day or 14-day time periods. Damages were assessed accordingly. The parameters that were taken were quantifying: the number of fruits that were prematurely dislodged, number of stylet sheaths that were present on each fruit, weight of each fruit (g), number of fruits containing deformation, number of fruits showing fungal signs (brown rot *Monilia laxa*), number of potentially marketable fruits, and the yield (g) of each sample. Exposure of the fruits to BMSB infestation resulted in decreased fruit weight, increased number of stylet sheaths, increased number of cherries with signs of fungal infestation, decreased number of potentially marketable fruits, and decreased yield. Increased period of time exposure resulted in decreased number of potentially marketable fruits, increased number of fruits with fungal infestation, and decreased yield. Results showed that the impact of the BMSB can be high on cherry, with crop losses occurring at low infestation density. The BMSB is a serious pest of cherry and management actions may be necessary to avoid economic losses.

Keywords: Cherry, damage assessment, *Halyomorpha halys*

PO036

THE PREVENTION OF BIOLOGICAL INVASIONS: THE CRUCIAL ROLE OF IMPORT CHECKS AT BORDER INSPECTION POSTS (BIPS). SOME CASE STUDIES

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The increased international trade with living plants and plant-based products creates possibilities for introduction of organisms to new areas, where they may develop to invasive pests. Italy is particularly exposed due to its exceeding points of entry, geographical position, habitat patterns. Campania Region contributes to the strengthening of controls on imported vegetal goods from Countries not belonging to EPPO region by its Plant Health Service. Goods reaching the BIPs are meticulously inspected, supported by scientific skills of partners involved in the operations (through URCoFi Project). Although many of these goods are recognized as pest-free, several species potentially harmful to our agro-ecosystems were stopped in the last years. Some case studies are reported: At the port of Naples annually, several containers are rejected and sent to the origin because of the infested contents. 1) Inspection on *Ficus microcarpa* from China highlighted the presence of the agaonidae *Josephiella* sp., soft scales *Ceroplastes floridensis* and *Lecanium* sp., while roots were attacked by *Meloidogyne* sp. nematodes. 2) An iconic case is represented by the finding on abachi Cameroonian (*Triplochiton* sp., although was a non-regulated product) of numerous arthropods and pathogenic fungi, which constituted almost an entire ecosystem. 3) In the recently set-up airport phytosanitary post, a Ghanaian passenger's baggage contained some *Solanum aethiopicum* fruits. Several of these fruits showed some holes probably ascribable to carpophagous parasites and laboratory observations allowed recovering some *Lepidoptera larvae*. Molecular analyses on larvae showed the specimens belong to *Leucinodes africensis* (Lepidoptera: Crambidae), a pest of Solanaceae absent in EPPO region. The aforementioned cases show that several interceptions occur every year and that adequate actions (inspections, training courses, information campaigns, agreement with other border institutions) and involvement of human and financial resources can guarantee a prompt and proficient prevention, thus preventing biological invasions by appropriate and timely risky material destruction.

Keywords: Alien pest, biological invasions, plant inspection, point of entry

PO037

STORAGE PESTS, ORIGINS AND DISTRIBUTIONS: A QUATERNARY PERSPECTIVE

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Work on fossil insect faunas from human settlements and records of pests of stored products provide an insight on the spread of species of pests and an understanding of the initial itineraries which led to their current global distributions. This paper will provide an overview of introductions of a few of the main pests to Europe with the initiation of farming, including *Sitophilus granarius*, *Oryzaephilus surinamensis* and *Tribolium castaneum*. It will include discussion of the beginnings and spread of agriculture and the role of the movement and establishment of storage pests to new areas for understating cultural transitions and synanthropic change. Discussion will centre upon significant periods for the spread of pests and their implications. The expansion of the Roman Empire and the feeding of its armies, Norse colonisation, and trade in the medieval and the post-medieval period will be considered from the biogeography of the pest records. The importance of human impact as a driver for the spread of synanthropic faunas and their toll on past societies will be discussed. In addition, preliminary studies from North America will be presented in an effort to understand differences and similarities between the two hemispheres.

Keywords: Fossil insects; storage; biogeography; Holocene; archaeoentomology

PO038

THE ALIEN PLANTHOPPER *RICANIA SPECULUM* ACQUIRES FLAVESCENCE DORÉE PHYTOPLASMA, BUT IS UNABLE TO TRANSMIT IT

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Ricania speculum is an alien planthopper accidentally imported from Far East, detected for the first time in the province of Genoa (Liguria, Italy) and spread out in Liguria and Tuscany regions. The species is univoltine in Italy, its overwintering eggs are inserted in linear rows inside thin plant structures (*e.g.* twigs, leaf ribs, stems). The nymphs have a huge wax tail used like a parasol for covering their bodies when disturbed. *Ricania speculum* is polyphagous on wild and cultivated plants, herbaceous and woody crops. This phloem feeder colonizes, among others, grapevine and Clematis vitalba plants, well-known hosts of Flavescence dorée phytoplasma (FDp), a quarantine phloem-limited pathogen, transmitted by the Nearctic leafhopper *Scaphoideus titanus*, which is one of the main constraint to viticulture in several areas of Europe. Moreover, two Ricaniidae specimens tested positive for the Banana Wilt Associated Phytoplasma (BWAP) in Papua New Guinea. Therefore, the aim of this work was to ascertain if *R. speculum* could act as a vector of FDp. To this purpose, nymphs reared from eggs were allowed to feed on FDp-infected broad beans and, after the completion of the latent period, transferred onto grapevine, broad bean and *C. vitalba* test plants for inoculation. At the same time, a number of adults fed on infected source plants were analyzed by PCR for the presence of FDp to check if the planthopper was able to acquire the pathogen. Transmission experiments to about 50 test plants and PCR analysis of more than 60 adults showed that *R. speculum* is unable to transmit FDp, although some individuals can acquire the phytoplasma and support a low level of its multiplication, as shown by qPCR. In conclusion, although *R. speculum* can damage plants by feeding and egg laying, it should not be regarded as a threat for FDp spread

Keywords: Insect vectors; Grapevine; Clematis vitalba; Ricanidae

PO039

APHROPHORIDAE (HEMIPTERA) VECTORS OF *XYLELLA FASTIDIOSA PAUCA* OQDS JUVENILE QUANTITATIVE SAMPLING

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Few Aphrophoridae, namely *Philaenus spumarius*, *P. italosignus* and *Neophilaenus campestris* transmit *Xylella fastidiosa pauca* ST53 causing the Apulian OQDS episode. Vector control is the effective, least impacting and sustainable action to manage and contain insect-borne pathogens. One of us (Porcelli) presented the first draft of a Aphrophoridae vector control strategy during the Symposium in Gallipoli. The same author proposed a more elaborated scheme for EFSA and in POnTE H2020 project kick-off. The strategy targets juvenile spittlebugs for vector control also because they are out of the olives trees, unable to transmit and far in time from transmission risks, pretty delicate and susceptible to several control means, relatively easy to count ex ante and ex post control action, with a control window wide enough to allow a second chance in case of first control action failure. Vector population size is one of the few key factor needed to shape a rational control strategy, but traditional adult sweeping net sampling or juvenile seeking by a looking glass do not gather the needed quantitative data. From 2016 we faced the need for novel sampling techniques focused on juvenile spittlebugs because naiades and nymphs are into the spittle and almost immotile, thus appearing the best possible instars for population measure. First experiments by flotation consisted in collecting herbs in olive orchards, either managed or abandoned, and in urban areas from within a transect and to wash the plants in water until any further juvenile was sieved out from the bath. Reducing the transect size from 0.25 to 0.04 m2 and adding NaCl and Sucrose to increase the juvenile floating we suggest a method simple and effective that recorded the highest Aphrophoridae juvenile population (about 10,470,000 per ha). Flotation demonstrated striking differences in population among managed, unmanaged groves and urban environments.

Keywords: Xylem-sap feeders, xylematic bacteria, alien invasive pest, plant disease, Mediterranean, CoDiRO, AcquaSamPling, Italy

PO040

ENVIRONMENTAL NICHE UNFILLING AND DIET NICHE SHIFT IN THE INVADED AREA BY THE SOLITARY WASP *ISODONTIA MEXICANA* (HYMENOPTERA: SPHECIDAE)

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One of the important questions in invasion ecology is to evaluate if a alien species has occupied the same native ecological niche in the invaded areas (niche conservatism), if it has occupied only a part of it (niche unfilling), or if it has changed its niche (niche shift). Here, we tested these three alternatives of niche occupation pattern in the American wood-nesting, solitary wasp, *Isodontia mexicana* (Hymenoptera: Sphecidae), which was first detected as alien species in France in 1960 and now present in most Europe. In particular, after having updated the current distribution of *I. mexicana*, we estimated 1) the environmental space occupied in both native and invaded range, subsequently predicting its potential global distribution, and 2) the diet niche (prey use) occupied in both native and invaded range. The wasp lives in areas of the Northern hemisphere with moderate and stable temperature, rather humid, and at low altitudes. The environmental space occupied in the invaded areas is included in, but does not overlap with, that occupied in the native areas, suggesting a process of niche unfilling of the invaded range. On the other hand, the wasp, which is restricted in hunting only gryllids and tettigonids, tends to maintain its prey preference in the invaded areas, albeit also extending the prey spectrum, suggesting niche shift. *I. mexicana* could also live in other temperate areas, mainly in the Southern hemisphere, particularly on the coasts. However, geographic (oceans) and/or climatic (*e.g.* mountain chains) barriers would allow the species to reach these potential areas only through human trade activity. Thus, *I. mexicana* could still occupy new areas by active dispersion, but confined to Europe. If new introductions will occur in extra-European areas, the species would easily find suitable prey, given the potential to expand its diet spectrum within the two hunted orthopteran families.

Keywords: Environmental niche, BIOCLIM, prey use, wasp, invasion

PO041

THE PROJECTALIEM "ACTION POUR LIMITER LES RISQUES DE DIFFUSION DES ESPÈCES INTRODUITES ENVAHISSANTES EN MÉDITERRANÉE" IN SARDINIA

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Human activity and global trading have promoted biological invasions of exotic pests in new environments, which is an important cause of biodiversity loss. In this framework, the project ALIEM "Action Pour Limiter les risques de diffusion des espèces introduites Envahissantes en Méditerranée" involves institutional partners from five territories of France and Italy and aims to develop adequate instruments for early detection of invasive pests and a cross-border observatory for flora and fauna. The model invasive species studied in order to achieve the goals of the project are the European hornet, *Vespa crabro*, the Asian hornet also known as yellow-legged hornet, *Vespa velutina*, and the red palm weevil, *Rhynchophorus ferrugineus*. The Asian hornet is a honeybee predator originating from China that was introduced accidentally into Europe in 2004 and at the moment has not been reported in Sardinia. The European hornet has presumably established in Sardinia since 2010. The monitoring of both species in required in view of the potential impact of *V. velutina* on honeybees and other social hymenopterans, also considering that the introduction of alien generalist predators may impact the food-web by changing the inter-specific relations through predation and/or competition. For this reason, the relationship between prey (*Apis mellifera ligustica*) and predator (*V. crabro*) will be evaluated through behavioral observations in an apiary context. The red palm weevil is a major pest of palms that has established in Europe in the '90. It has been recently observed also in the dwarf palm, *Chamaerops humilis*, the only native West Mediterranean palm species. In view of the ecological importance of *Ch. humilis*, extensive monitoring on cultivated and wild dwarf palms has been carried out, together with behavioral experiments.

Keywords: *Vespa crabro*, *Vespa velutina*,*Chamaerops humilis*, alien species

PO042

OVERWINTERING IN INVASIVE LADYBIRD *HARMONIA AXYRIDIS*: THE EFFECT OF WINTER TEMPERATURE ON SURVIVAL AND BODY MASS LOSS

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Harlequin ladybird, *Harmonia axyridis*, is considered among the most successful invasive insects worldwide. Probable traits that predetermine its success are dominance in intraguild predation, efficient chemical defence and excellent immune system. However, high winter survival could be another potential advantage of this species. In this study, we investigated the effect various temperature courses during winter (mild, standard and harsh winter) on winter survival, body mass loss and spring longevity for individuals reared under laboratory conditions and these collected in nature during October. Beetles were individually overwintered in Petri dishes placed in computer-instructed climatic chambers mimicking real temperatures courses recorded in nature (based on long-term data collected at meteorological station situated in Prague-Ruzyně, Czech Republic). Beetles that survived the winter part of the experiment were subsequently involved in experiment measuring their early spring longevity at 20°C without any food. In general, winter survival of beetles reared under laboratory conditions was much lower than survival of beetles collected in nature. Lower winter temperatures decreased survival rate for beetles with laboratory as well as field origin. Body mass loss was significantly higher under regime of mild winter compared to standard and harsh winter conditions and body mass loss was significantly higher in beetles of laboratory origin than in two out of three populations of beetles collected from nature. Early spring longevity was lower in laboratory breed beetles than these collected in nature. Ladybirds which survived harsh winter regime were able to survive for a longer period without any food in early spring than beetles exposed to a warmer regime (harsh >standard >mild). Overall our results indicate that lower winter temperatures cause enhanced mortality in *H. axyridis* due to chill injuries, but warmer winter temperatures results in depleted energy reserves and thus lowered performance during early spring season.

Keywords: Biological invasion, body mass, *Harmonia axyridis*, overwintering, survival, temperature

PO043

MONITORING OF *HALYOMORPHA HALYS* (HEMIPTERA: PENTATOMIDAE) IN THE URBAN AND PERI-URBAN ENVIRONMENT OF GENEVA, AND IMPACT OF HEAT WAVES ON ITS DEVELOPMENT

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Halyomorpha halys was first observed in Switzerland in 2007, in the canton of Zurich. Since its introduction, this pest is now present in several cantons, but it is just recently that damages were observed on fruit crops. To better anticipate the expansion of this pest in Geneva, a project was realized from 2013-2017 in order to: 1) detect *H. halys* along an urban-rural gradient, 2) evaluate the impact of environmental factors on its presence and distribution, and 3) measure the impact of heat waves on its survival and development. Several sites along an urban-rural gradient were monitored from May to November in Geneva. Results obtained from this monitoring were then analyzed to establish correlations with some factors known to influence the presence and abundance of *H. halys*. For the impact of heat waves on *H. halys*, eggs and larvae were exposed to a heat wave treatment compared to a control in climate chambers. Survival and time of development were recorded after 10 and 15 days. Monitoring results showed that *H. halys* is still mainly found in urban areas compared to rural ones. However, individuals were found for the first time in 2017 in different crops and in a tree nursery in the peri-urban area. Differences were observed between urban and rural sites regarding the time that *H. halys* appeared in the season and its abundance. Among environmental factors analyzed, proportion of hard surfaces is highly correlated with the total number of *H. halys* found in traps as well as the temperature and the distance from a known urban infestation spot. Survival of eggs and larvae exposed to heat waves are not different than the control. However, there was a significant difference between the two treatments for the time of development with a faster development of L2 exposed to heat waves.

Keywords: Integrated pest management, climate change, insect biology, agriculture, urban habitat

PO044

BIOLOGICAL TRAITS OF THE RED-NECK LONGHORN BEETLE *AROMIA BUNGII* (FALDERMANN) (COLEOPTERA: CERAMBYCIDAE)

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The red-neck longhorn beetle *Aromia bungii* (Faldermann) (Coleoptera: Cerambycidae), is a xylophagous pest of stone fruit trees native to East Asia. The species was accidentally introduced in Italy (Campania Region) before 2010, but its presence was formalized only in 2012 following the record of massive infestations on numerous *Prunus* sp. trees. *A. bungii* attacks healthy plants, where the larvae bore galleries under bark, in the sapwood and heartwood of trunk and main branches, weakening the whole plant due to the interruption of the lymphatic flows. Field samplings conducted in collaboration with the Regional Phytosanitary Service, soon highlighted that *A. bungii* may represent a serious threat to stone fruit crops, and therefore the alien pest was first added to the EPPO Alert list and in 2014 to the EPPO A1 list. Due to the lack of reliable data about *A. bungii* biology, since 2015 laboratory studies were carried out to improve knowledge on life history and to program its adequate IPM. Different biological parameters such as the longevity of both sexes at two different temperatures and food supplies, ovigeny index, daily oviposition, realized fecundity and fertility were investigated. A possible correlation between body size and fecundity of females was assessed. The maximum longevity was reached by fed males at 20°C while starved females at 25°C showed the shortest life span. *A. bungii* had an ovigeny index of 0.6 showing its pro-ovigenic status and the oviposition period lasted up to two months. Females laid in average 450 eggs with hatching rate above 70%. The largest amount of eggs was laid during the first week after mating. No correlation body size/fecundity was found. Obtained results suggest that the high biotic potential of *A. bungii* can explain the establishment in short time of a harmful population in the outbreak area of southern Italy.

Keywords: Fecundity, invasive species, laboratory observations, longevity, stone fruit

PO045

DETERMINATION THE EFFECT OF CHITOSAN AND NANO CHITOSAN ON *PEGOMYIA HYOSCAMI* (WITT) (DIPTERA: ANTHOMYIDAE)

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Sugar beet is considered one of the most important crops in Egypt because it was used in the production of sugar as it contains 15-20% sugar. *Pegomyia hyoscami* (Witt) (Diptera: Anthomyidae); harmful insect pest causing a lot of damage of the sugar crop. Chitosan (CS)-g-poly (acrylic acid) (PAA) nanoparticles, which are well dispersed and stable in aqueous solution have been prepared by template polymerization of acrylic acid in chitosan solution which have an insecticidal effect on insect pests. The usage of chitosan and nano chitosan test against *P. hyoscami* Results showed that, the LC50 obtained 144 and 70 ppm after *P. hyoscami* treated with different concentrations of chitosan and nano chitosan. Also, under field conditions when *P. hyoscami* treated with the chitosan and nano chitosan, the number of eggs significantly 38±5.7 and 1±6.7 eggs/ female as compared to eggs /female in the control. The percentage of egg hatching, larval mortality, malformed pupae and malformed adults significantly decreased in case of chitosan treatments and almost reduced after nano chitosan treatments. The weight of sugar beet significantly increased to 2386±35.13 and 2470±54.28 kg/ feddan as compared to 1750±33.46 1210±85.89 kg/ feddan in the control during season 2016 1nd 2017 respectively.

Keywords: Nano, chitosan, *Pegomyia hyoscami*, sugar beet, control.

PO046

TOXIC EFFECT OF CHITOSAN AND NANO CHITOSAN ON *PHYLLOTRETA CRUCIFERAE* (GOEZE) (COLEOPTERA: CHRYSOMELIDAE)

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Sugar beet is considered one of the most important crops in Egypt because it was used in the production of sugar as it contains 15-20% sugar. *Phyllotreta cruciferae*(Goeze) (Coleoptera: Chrysomelidae); harmful insect pest causing a lot of damage of the sugar crop. Chitosan (CS)-g-poly (acrylic acid) (PAA) nanoparticles, which are well dispersed and stable in aqueous solution have been prepared by template polymerization of acrylic acid in chitosan solution which have an insecticidal effect on insect pests. The usage of chitosan and nano chitosan test against *P. cruciferae*. Results showed that, the LC50 obtained 140 and 72 ppm after *P. cruciferae* treated with different concentrations of chitosan and nano chitosan. Also, under field conditions when *P. cruciferae* treated with the chitosan and nano chitosan, the number of eggs significantly 43±4.7 and 1±8.4 eggs/ female as compared to eggs /female in the control. The percentage of egg hatching, larval mortality, malformed pupae and malformed adults significantly decreased in case of chitosan treatments and almost reduced after nano chitosan treatments. The weight of sugar beet significantly increased to 2387±50.11 and 2478±78.68 kg/ feddan as compared to 1750±51.23 1223±75.79 kg/ feddan in the control during season 2016 and 2017 respectively.

Keywords: Nano, chitosan, *P. cruciferae*, sugar beet, control.

PO047

EFFECTS OF 5-AZA-2´-DEOXYCYTIDINE ON EGG FECUNDITY OF *ACHROIA GRISELLA* F. (LEPIDOPTERA: PYRALIDAE)

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The nontarget effects of 5-Aza-2´-deoxycytidine (5-Aza-dC), an epigenetically-effective agent, were assessed on the adult fecundity and fertility of F1 females of the smaller wax moth, *Achroia grisella* F. (Lepidoptera: Pyralidae), one of the economically important pests of wax in the world. Different doses of 5-Aza-dC (0.1, 0.5, 0.75, and 1.0 mg/ml) were added into the insect diet as water source in all bioassays and chemical-free diet were used as controls. The total number of viable and damaged eggs based on three daily results noticeably decreased on the first, second, and the third day. However, the decline was significant only at 1.0 mg/ml dose for viable eggs on the third day and for damaged eggs on the first day according to control. The reducing effect of the chemical on egg numbers was especially important at higher doses (0.75 and 1.0 mg/ml) based on three-day observations. Similarly, 5-Aza-dC also caused a prominent reduction in the number of unhatched eggs (dead larva, unfertilized and damaged eggs) in general (especially significant at 0.5 and 1.0 mg/ml doses for dead larvae). Moreover, 5-Aza-dC elicited a sharp decrease on% fecundity at >0.1 mg/ml dose compared to control however the effects on% fertility was to a lesser extent. Therefore, it is obvious according to the current results that 5-Aza-dC had an adverse activity on the reproductive potential of *A. grisella* based on daily and three-day observations across trophic levels. The insignificant, but the high value of corrected% sterility at 0.75 and 1.0 mg/ml doses according to others was also supporting this inference. The stress-induced, trophic interaction of 5-Aza-dC seems to lead above mentioned adverse effects on fecundity of *A. grisella*. From an evolutionary point of view, the frequent usage of chemicals is still considered a danger to the continuity of the species.

Keywords: *Achroia grisella*, 5-Aza-2´-deoxycytidine, number of eggs, fecundity, reproductive potential.

PO048

Poster withdrawn.

PO049

EFFECT OF HIGH AND LOW TEMPERATURES ON THE MORTALITY OF BROWN MARMORATED STINK BUG, *HALYOMORPHA HALYS* (HETEROPTERA: PENTATOMIDAE)

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Extreme high and low temperatures can have dramatic effects on insect survival, but this effect can be different depending on the physiological status of the insects. Here we evaluated the effect of exposure to high and low temperature on adults of Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys* (Stål, 1855), an invasive pest in Europe. Using a thermostats, field collected adults were exposed to stable temperature regimes for different exposure time. In particular, overwintering and overwintered adults were exposed to low temperatures (from -2.5°C to -14°C), while overwintered and summer developed adults were exposed to high temperatures (from 35°C to 42.5°C). Exposure times were from 1 to 4 hours. Mortality curves at different timextemperature exposures were estimated. In low temperature regime, BMSB starts to die with temperature lower than -5°C and after 2 hours of exposure. At high temperatures, BMSB mortality was observed at temperature higher than 40°C and with 1 hour of exposure. The effect of temperature was influenced by physiological status: overwintered and summer adults were more resistant to low and high temperature, respectively.

Keywords: *Halyomorpha halys*, Extreme temperatures, Mortality

PO050

INTERACTION BETWEEN *HALYOMORPHA HALYS* STÅL AND ITS HOST PLANT: INDUCED DEFENSE AND FEEDING BEHAVIOR

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Halyomorpha halys Stål (Heteroptera, Pentatomidae), the Brown Marmorated Stink Bug (BMSB), is native to Eastern Asia, where it feeds on a large diversity of host plants. BMSB has been accidentally introduced in Switzerland, Europe, where first observations occurred in 2007. It is probable that the pest will have colonized a large part of Europe within the next decades. It is therefore crucial to better understand its biology to develop efficient control strategies. *Phytophagous Pentatomidae* use different feeding strategies according to the plant tissue. On seeds, they apply a cell rupturing strategy, while on leaves and stems, they secrete a salivary sheath to facilitate the penetration of the stylets through the cells. Regarding their feeding strategies, they are more likely to induce mainly the jasmonic acid pathway as a plant defensive response. Yet, there is a lack of knowledge concerning the behavioral and physiological response of an insect exposed to an elicited plant. Therefore, we aimed to enlighten the interactions between BMSB and one of its host plant, focusing on: (1) the validation of the hypothesis that jasmonic acid pathway is induced by this insect feeding; (2) whether other individuals are subsequently able to detect that response and adapt their feeding strategies or salivary compounds. Our results highlighted a local and systemic defensive response in leaves after an attack by BMSB or after its saliva injection. Furthermore, we proposed a first description of electrical penetration graph waveforms for that pest. Then we showed some differences in feeding behavior parameters and in salivary compounds according to the plant treatment. We replaced these results in the context of such a polyphagous invasive species and its ability to overcome the plant defense to successfully settle and feed.

Keywords: *Halyomorpha halys*, plant defense, electrical penetration graph, feeding behavior

PO051

INFLUENCE OF DIETARY YEASTS ON THE FECUNDITY OF ADULT SPOTTED-WING DROSOPHILA (*DROSOPHILA SUZUKII*)

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The spotted-wing drosophila (*Drosophila suzukii* Matsumura; Diptera: Drosophilidae), native to Asia and widespread throughout North America, South America and Europe, is an invasive insect pest. The tendency of *D. suzukii* to lay eggs in healthy ripe fruits makes it an important insect pest for soft and stone fruits. Several studies document the attractiveness of yeast volatiles to *D. suzukii* adults and the important role of yeasts in the diet of *D. suzukii* larvae, suggesting that *D. suzukii* adults are also affected by the yeast community in their diet. Under laboratory conditions the effects on the fecundity and mortality, of eight different epiphytic yeast species offered in the diet of *D. suzukii* adults were observed over a test period of 50 days. Among the yeast species known to be associated with *D. suzukii*, the ones used in this study turned out to increase the number of eggs laid compared to the yeast-less control (malt extract agar). The number of eggs laid, the survivorship, and egg laying behaviors of *D. suzukii* adults were observed simultaneously for each yeast species offered. Significantly more eggs were laid by females fed with baker's yeast and *H. uvarum* compared to females fed with other yeast species. *D. suzukii* females showed a significantly shorter lifetime when fed with yeast species which lead to higher fecundity. The eight yeast species showed different results when grown on potato dextrose agar, suggesting an influence by the culture medium on the yeast-*D. suzukii* interaction. The beneficial nutritional effect of the yeast species *H. uvarum* on the fecundity of *D. suzukii* was observed on both culture media. Investigations are ongoing to understand the reason of the mutual interaction between specific yeast species and *D. suzukii* adults.

Keywords: *Drosophila suzukii*, spotted wing drosophila, yeast, fecundity

PO052

ESTERASE ACTIVITY IN *OSTRINIA NUBILALIS* LARVAE UNDER *in vitro* CONDITIONS

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The European corn borer (*Ostrinia nubilalis* Hübner) is a crop pest that feeds on a wide range of host plants including dicots and is considered one of most serious maize pest in many parts of Europe also in Poland. Pest organisms should at first be controlled by biological methods and, if these are ineffective, the use of chemical agents is allowed. The research on corn borer in Poland is mainly focused on its spread monitoring and ways of pest control. In order to increase the effectiveness of this control, it is necessary to analyze the activity of detoxification systems in this species. One of the elements that allows insects to survive treatment with insecticides is the activity of non-specific esterases that hydrolyze xenobiotics. In this study esterase activity in the last stage larvae (L5) of *Ostrinia nubilalis* under *in vitro* conditions was analysed using a spectrophotometric assay and native polyacrylamide gel electrophoresis. We undertook optimalization reaction of non-specific esterases in the substrate concentration range under different pH conditions and analysis of the differentiation of esterase allozymes against selected substrates. We found that the highest esterase activity in the *Ostrinia nubilalis* larvae measuring p-nitrophenyl acetate hydrolysis was at pH 7.8 at a concentration of 0.59 mM. Analysis of the native PAGE zymograms revealed substrate dependent number of band and their distribution. Depending on the substrate used, the isozymes were characterized by different activities, with a greater affinity for the α forms than the β of the esters used. In addition, esterases revealed greater affinity for compounds having a two-carbon than a three-carbon chain. Such research gives the opportunity to analyse the response of insects to the plant protection products which may influence the effectiveness of pest control.

Keywords: *Ostrinia nubilalis*, esterases, activity, native PAGE

PO053

THE IMPACT OF THE PARASITOID WASP *ANASTATUS* SP. ON BAG-SHELTER MOTH (*OCHROGASTER LUNIFER* HERRICH-SCHÄFFER) POPULATIONS

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Bag-shelter moths (*Ochrogaster lunifer* Herrich-Schäffer) are common and widespread across coastal and inland Australia. *Ochrogaster lunifer* larvae have urticating setae that cause medical problems in humans, including urticaria, dermatitis and severe allergic reactions. *Ochrogaster lunifer* is an important species in equine health due to its role in miscarriages when setae are accidentally ingested by pregnant mares. Adult *O. lunifer* emerge in October - November, and mated females deposit an egg mass consisting of 150-550 eggs on the host tree. These eggs are covered with scales from the female moth's anal tuft, which are urticarial and provide protection from natural enemies. Investigating mortality rates in *O. lunifer* is essential to understanding their population dynamics. Common causes of mortalities include egg parasitism by chalcid wasps (*Anastatus* sp.), egg and first instar predation by dermestid adults and larvae, and larval parasitism by tachinid flies. Egg parasitism in *O. lunifer* is the focus of this presentation. Egg masses were collected from The University of Queensland (UQ), Gatton campus, Queensland, Australia (-27°56'S, 152°34'E) at different time intervals – some egg masses were collected at initial sighting, as well as after two and four weeks later. Egg masses were kept in the lab for 28 days, and then frozen to terminate development. Egg masses were preserved in 70% ethanol and scrutinised under a stereomicroscope to determine the fate of each egg in the egg mass. Understanding the life history of *Anastatus* sp. may open possibilities for its application in pest management for *O. lunifer*.

Keywords: Processionary caterpillars, Parasitoid and host interactions, Population dynamics

PO054

PHEROMONE TRAPS FOR *HALYOMORPHA HALYS* MONITORING: EVALUATION OF SIDE-EFFECTS IN FRUIT ORCHARDS

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The brown marmorated stinkbug *Halyomorpha halys* (Stål) (Heteroptera, Pentatomidae) is an invasive polyphagous alien species detected in Italy for the first time in 2012, in the Modena province. By piercing and sucking on fruits and seeds, it causes damages that render products unmarketable. A few years following its discovery, it is reported as a key pest of fruit orchards all over Northern Italy and is presently threatening many agricultural crops in other invaded European countries. Currently, the monitoring of *H. halys* is performed using traps baited with specific aggregation pheromones combined with a synergist. The stinkbugs respond to the lures by aggregating both inside and outside the traps. The presence of individuals feeding actively in the area around the trap, with a consequent increase in fruit damages, represents a side-effect of this type of traps, which poses a strong limitation for their use in commercial fruit orchards. The aim of this study was to assess the range of side-effects around the traps in fruit orchards. A field experiment was performed in 2016-2017 to estimate the area around the traps potentially affected by the increase in fruit damage. A baited trap was installed in the first row of selected pear orchards at the beginning of May and the percentage of deformed fruits on adjacent plants was evaluated one week before harvest. The results show that fruit damage was particularly high on plants located within 4-5 m from the traps, while an acceptable level of damage was found on plants situated more than 10 m from the traps. These results provide fundamental information for the use of traps baited using aggregation pheromones in the field.

Keywords: Brown marmorated stink bug, aggregation pheromones, pear orchard, fruit damage, invasive species

PO055

RANGE EXPANSION OF *METCALFA PRUINOSA* (HOMOPTERA: FULGOROIDEA) IN BULGARIA

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After its introduction in Europe in the late 1970's the citrus flatid planthopper *Metcalfa pruinosa* (Say), a Nearctic species of Fulgoroidea has spread over most of Europe. This extremely polyphagous species was reported for the first time in Bulgaria in 2004 on *Thuja oxidentalis* L. in small numbers in restricted urban area. Feral populations of *M. pruinosa* were found in 2009 at the Northern part of Black sea coast on *Acer campestre* L. and *Robinia pseudoacacia* L. In 2010 the species was detected at same localities only. During next several years *M. pruinosa* has spread over the Black sea cost of Bulgaria reducing dramatically the ornamental value of urban vegetation. During the period 2016-2017 more than 50 sites covered by semi natural vegetation and ornamental plants were surveyed in cities and their surroundings, to assess the presence of *M. pruinosa* and its parasitoid *Neodryinus typhlocybae* (Ashmead) in Bulgaria. The pest was detected in regions of Sandanski, Petrich, Sofia, Dobrich, Dolni Dubnik and Pleven and cities along the Black sea coast, but heavy infestation was observed in urban areas along Black sea coast and Pleven only. *Neodryinus typhlocybae* was detected in region of Varna and Dobrich. Our results suggest that the planthopper is still in the expansion phase of the invasion process in Bulgaria. The pathways of introduction of species are discussed.

Keywords: alien, insecta, parasitoid, distribution, host Bulgaria *Metcalfa pruinosa*

PO056

RELIABLE HOLD ACROSS THE GLOBE? - A CASE STUDY ON SOUTHERN GREEN STINK BUGS

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The southern green stinkbug *Nezara viridula* (Heteroptera, Pentatomidae) is cosmopolitan and highly polyphagous, attacking more than 150 species of dicotyledonous plants and several monocots, showing a preference for legumes. It temporarily switches between plants, because the host preference changes with host maturity and phenology. *N. viridula* spreads all over the world; thus, beeing a representative model species in studies on the impact of global warming on insect populations. Thus, this stink bug must successfully overcome the evolutionary attachment hurdle. How does it access the variety of host plant substrates? The present study focuses on the hold of the southern green stink bug on its host plants. Both robust claws and tough adhesive pads enable the *N. viridula* bugs to grasp and adhere to a wide range of various plant substrates, including such ones covered with anti-adhesive wax crystals. Traction force experiments with living adult stink bugs were carried out. Tabular, pruinose pea leaves were detected to prevent the foothold of the southern green stink bugs, although pea is known as one of their common host plants.

Keywords: Anti-adhesive, cuticle, epicuticular wax crystals, insect attachment, pest management, plant surface

PO057

HOST-PARASITOID PHENOLOGY AT TWO DIFFERENT ALTITUDINAL GRADIENTS, THE CASE OF THE CHESTNUT GALLS WASP IN A NEW EXPANSION AREA

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Parasitoids adaptation to a new host is related to their own phenology and it's closely connected to the thermal trend of their diffusion area. We studied phenology and interactions of Asian chestnut gall wasp (ACGW) with native parasitoids in two altitudinal areas in southern Italy. We were able to develop phenology models to describe timing of cynipid stages (larvae, pupae, adults and adults emergence) across all sites and years, as a function of accumulated degree-days. Our results showed that these models were responding more accurately to the prediction where they were developed. Parasitoid species richness was different among the different areas and a minor number of species was recorded at lower altitude. The phenology estimator used (average of the occurrence days weighted by its abundance on each date) to compare the same parasitoid species at two different altitudes made evident that the reduction of the developmental time of some ACGW host stages (larvae and pupae-adults) makes parasitoids less flexible in their use as resource. The timing comparison between ACGW preimaginal and adult stages and the specific parasitoid complexes, stressed that the shortest period of occurrence of cynipids in higher temperature areas has a negative effect on the host-parasitoids relationship favoring a decoupling of phenology for some parasitoids. Species as *Sycophila variegata*, *Megastigmus dorsalis* and *Torymus auratus* show off a reduced parasitization to ACGW at higher thermal conditions. Overall, these results suggest that both host phenology and parasitoids species are affected in a similar way by temperature, but that ACGW is faster than parasitoids species in accelerating phenology in response to spring and summer warming. The findings underline how the parasitoids adaptation process to new host is limited by the short duration of host presence in the galls and could explains the considerable variability in the parasitization of the cynipid gall wasp at the different altitudes where chestnut is distributed.

Keywords: Parasitoids, cynipids, phenology, temperature

PO058

AN INTEGRATIVE APPROACH TO ASSESS THE TAXONOMY AND PHYLOGEOGRAPHY OF THE GENUS *LIOPHLOEODES WEISE* 1894 (COLEOPTERA:CURCULIONIDAE)

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Liophloeus (Coleoptera:Curculionidae) is a genus of weevils consisting of two subgenera: Liophloeus sensu stricto (including sexual and parthenogenetic taxa with wide range of ecological preferences) and Liophloeodes (including only sexual taxa, connected with wet and cold biotopes, occurs mostly in mountains). Traditional taxonomy of this group is rather poor. Liophloeodes species share similar ecological preferences and almost identical morphology, apart from the shape of aedeagus (part of male genitalia), which is the traditional diagnostic trait for species delimitation. Also their geographical ranges are partially overlapping, however every species has its own distribution pattern connected with mountain massifs- different parts of Carpathians, Alps, Dinaric Alps or other mountains. Therefore, they could be young species or populations under speciation, and their evolution may have been shaped by processes connected to the last glaciation and interglaciation events. It is possible that such species are currently in the "warm-stage" refugia, and the Carpathians (which are the center of Liophloeodes' range) are one of them. The aim of this study was to delimit species within the genus using an integrative approach based on molecular evidence [by sequencing two nuclear (28S-D2 and ITS2) and one mitochondrial (COI) markers] and morphometric evidence (by measuring several morphological traits). Topology of trees based on nuclear markers was fully congruent with the classification of studied specimens based on their genitalia. However, phylogeny of COI marker was only partially concordant with those results and suggested hybridization between some species. This hypothesis seems to be supported also by heterozygosity detected at nuclear loci. Moreover, results of morphometric study only indicated clear differentiation between Liophloeus sensu stricto and Liophloeodes, which may suggest that Liophloeodes species splitted recently.

Keywords: COI, hybridization, morphometry, nuclear markers, phylogeny, species delimitation, weevil

PO059

MORTALITY TESTS IN SPOTTED WING DROSOPHILA (*DROSOPHILA SUZUKII*) AT EXTREME TEMPERATURES FOR CALCULATING ESTIVATING AND HIBERNATING RATES

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The spotted wing drosophila (SWD) *Drosophila suzukii* is a pest of soft-skinned fruit with origin in Asia which invaded Germany in 2011 and became a serious pest in orchards and vineyards. The infestation intensity by the SWD in Germany in the last years showed that the fly's population size and therefore the damage are highly dependent on the climate. Predicting the SWD infestation risk by means of weather data would support guiding pest management strategies. Therefore we tested mortality related to extreme temperatures in the climate chamber with different life stages of *D. suzukii*. At temperatures between zero and -12°C within durations of two to 144 hours, we determined the cold tolerance of the summer and winter type of the adult flies. At temperatures between 36 and 42°C we investigated the heat tolerance of eggs, larvae and pupae (immobile stages). Statistical analyses indicated that the winter adapted flies were significantly more tolerant against cold than the summer type and that this difference was determined by the tested combinations of temperature and duration. The heat experiments showed a both-sided influence of temperature and duration relating to the mortality of all tested life stages. Pupae represented the most robust stadium. Eggs and larvae were more sensitive and quite similar in their heat tolerance what resulted in higher mortality rates. The results will be integrated in the decision support system 'SIMKEF' for a more precise calculation of estivating and hibernating rates within prognosticating infestation risk by the SWD in Germany.

Keywords: *Drosophila suzukii*, Spotted wing drosophila, cold tolerance, heat tolerance, decision support system

PO060

RADIOFREQUENCY MEDIATED LIFE-VARIABLES OF *CALLOSOBRUCHUS CHINENSIS* (COLEOPTERA: CHRYSOMELIDAE: BRUCHINAE) ON LEGUMINOUS SEEDS

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Callosobruchus chinensis (L.) is a destructive pest of stored products. Since chemical options have adverse effects on human health and environment, it is urgently needed for developing an environmentally friendly non-chemical method to replace chemical insecticides for managing stored products pests. We investigated the oviposition, development and adult performances of *C. chinensis* at five radiofrequency levels of 0 (control), 5, 10, 20, and 30 kHz on three leguminous seeds: azuki bean, cowpea, and mung bean. In addition, the effects were also studied in three successive generations (Parent, F1- and F2- generations) of *C. chinensis*. The results showed that radiofrequency application had significant effects on the life variables of *C. chinensis*. Radiofrequency exposures on *C. chinensis* not only impacted on developmental time, adult longevity and adult weight but negative effects were also transmitted to the successive generations. Oviposition was found to reduce as generation passed after exposed to radiofrequencies. Total developmental time from egg to adult emergence and adult longevity were longer and shorter in case of radiofrequency treatment than untreated, respectively. The lowest rate of adult emergence occurred at 5 kHz treatment. The outcomes of this study are discussed in terms of targeting susceptibilities to radiofrequency in storage and semi-field as an alternative to chemical treatments.

Keywords: *Callosobruchus chinensis*, Frequency levels, Adult performance, Semi-field, Non-chemical management

PO061

PHID-COLEO: A NEW DIAGNOSTIC TOOL FOR BEETLES INTERCEPTED IN WOOD PACKAGING MATERIAL

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The project PHID-Coleo (= Plant Health Identification of Coleoptera) has the objective to develop new diagnostic tools for the identification of potentially invasive and economically important beetles in wood packaging material. The identification methods include classical identification based on morphological characters as well as molecular methods based on DNA-barcoding by PCR. The methods for species identification will be supplemented by molecular analyses of introduced population to clarify within species variations. Such methods will make it possible to determine the taxonomic relationship of samples from different areas and to draw conclusions about the introduction pathways, resulting in more efficient monitoring of the invasive species and preventing their spread. Wood-breeding beetles introduced into Germany include the longhorn beetles *Anoplophora glabripennis* (Asian Longhorn Beetle) and *Aromia bungii* (Red-necked Longhorn Beetle). These and other longhorn beetles pose a high risk of economic damage to trees and wood products. Smaller beetles like the powderpost beetles from the families Bostrichidae and Lyctidae also have the potential for causing considerable damage but are often not identified adequately during inspections of wood packaging materials. This project will aim at closing that gap and to facilitate risk analyses of newly introduced species and for organising a fast reaction to such introduction and prevent their establishment and the resulting economic damage. PHID-Coleo will build a freely accessible database of relevant species which are potentially invasive, including morphological characters and data on genetic identification. The database can be used by plant protection services as well as commercial services and will also be available in a printed version. The development of methods for analysing population structures is an additional aspect which will be based on the Asian Longhorn Beetle as a suitable example. Another focus of PHID-Coleo is to build a collaborative network of museums, plant protection services and entomologists.

Keywords: Cerambycidae, Bostrichidae, Anoplophora, identification, key, plant health

New Approaches for the Management of Agricultural and Forest Pests

PO062

DIVERSITY AND ABUNDANCE OF DIURNAL INSECTS ASSOCIATED WITH DRY SEASON *AMARANTHUS HYBRIDUS* L. IN THE UNIVERSITY OF ILORIN, NIGERIA

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Amaranthus hybridus L. or green amaranth is an important leafy vegetable valued by resource poor farmers in Africa for its high nutritive value, early maturity and ease of cultivation even in the dry season. The productivity of *A. hybridus* in sub Saharan Africa is mainly limited by insect pest attack on the field. Integrated Pest Management (IPM) offers a sustainable alternative to synthetic chemicals for insect pest control within amaranth cropping systems. Amongst other objectives, IPM entails the conservation of beneficial insect species and the management of pestiferous ones. This study was thus carried out to provide information on the diversity and abundance of insect species associated with dry season *A. hybridus* during the day when farmers are most likely to come across them. Insect sampling with sweep nets was done on irrigated *A. hybridus* plots once a week for 8 weeks in the morning, afternoon and evening. Seventy seven (77) insect specimens consisting of 24 species, 16 Families and 5 Orders were collected during the study. Herbivores in the family Acrididae, Alydidae, Chrysomelidae and Pyrgomorphidae made up 78.3% of insect species collected in the morning. Insects like *Oecophylla* sp and *Dorylus* sp. in the family Formicidae were most abundant in the afternoon while parasitoids in family Sphecidae and leaf feeders like *Acrida bicolor* in family Acrididae made up 53.48% and 32.56% of total evening collections respectively. Insect taxa in the evening were significantly ($p<0.05$) less diverse than those of the morning or afternoon according to Shanon-Wiener diversity index (H') t-test. There was however no significant ($p>0.05$) difference in species richness or diversity indices of insect taxa collected in the morning and afternoon. It is hoped that information provided in this study will enhance insect biodiversity, sustainable pest management and ultimately improve green amaranth production.

Keywords: *Amaranthus hybridus*, diversity indices, diurnal insects, IPM, insect biodiversity

PO063

EFFECT OF DIFFERENT FODDER CROPS AND ARTIFICIAL DIET ON FECUNDITY AND SURVIVAL OF ARMYWORM, *SPODOPTERA LITURA FABRICIUS* (LEPIDOPTERA; NOCTUIDAE)

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The present study investigated the host suitability and its influence on development and fecundity of armyworm, *Spodoptera litura Fabricius* (Lepidoptera; Noctuidae) for devising an efficient strategy for the management of this economic pest. *S. litura* larvae were offered cabbage (*Brassica oleracea* L.), lucern (*Medicago sativa* L.) jantar (*Sesbania sesban* L.), maiz (*Zea mays* L.) and artificial diet. The third instar larvae fed on cabbage, lucern and jantar showed significant differences in their lengths (3.70, 3.43 and 3.10 cm respectively). While those fed on artificial diet and maize showed minimum lengths (3.07 and 2.96 cm). Similar trend was observed for weight of third instar larvae. Feeding duration was higher on cabbage, lucern and jantar (7.96, 7.60 and 7.50 days), while this duration decreased in case of artificial diet and maize (7.36 and 7.00 days). Prolonged survival rate (90%) was recorded on cabbage followed by lucern and jantar (69 and 53%). The declining trend in survival rate was observed when fed on maize and artificial diet (33 and 30%). Adult females laid more eggs in relation to lucern, jantar and cabbage (22, 21 and 16 egg batches) followed by artificial diet and maize (12 and 9 egg batches) with (10:10) sex ratio. The results revealed that *S. litura* preferred cabbage, lucern and jantar crops during life cycle. This information is helpful for the management of this notorious insect pest.

Keywords: *Spodoptera litura*, cabbage, lucern, jantarr, maize, artificial diet, larvae, pupae

PO064

AGE-DEPENDENT YIELD LOSS OF GREENHOUSE CUCUMBER INFECTED BY DIFFERENT DENSITIES OF VEGETABLE LEAFMINER, *LIRIOMYZA SATIVAE* (DIP: AGROMYZIDAE)

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The vegetable leafminer, *Liriomyza sativae* Blanchard (Dip: Agromyzidae), is a key pest of vegetable and ornamental crops worldwide and is mainly controlled by insecticides. Greenhouse studies were conducted to determine yield loss of cucumber, cucumis sativae (Cultivar Simenesis) by *Liriomyza sativae*. Effect of the pest density was investigated at four densities of 10, 20, 40 and 50 pairs of flies by including a control. Also three planting dates with one month intervals were included. A randomized complete block design (RCBD) were used in six replicates. Each block contained plants of different ages which infected by flies of one density any time. Greenhouse cages were used to separate blocks. The flies were permitted to oviposit for eight hours. Then the flies and cages were removed and three days later mines were located. Last instar larvae were removed at the end of development and as soon as pupae were hatched flies of the same number were released into reinstalled cages to begin a second generation. The mine number and area of destroyed tissue were measured individually by a transparent lattice at the end of leaf duration. Damage level was determined as cm^2.d (area of injured tissue×duration of damage). Fruits were picked up and weighed every 2-3 days. Relation between total mine number and area then were determined by linear regression. Mine area was determined as 184.2±12.0 mm^2 (n=3779). Taking 143.57 as variance estimate into account, 99.73% of mines had 148-220 mm^2 area. Also results revealed that both fly density and plant age had significant effects on cucumber yield ($P<0.000$). Younger infected plants respond more strongly than older ones. Also lower densities were tolerated by older plants.

Keywords: *Liriomyza sativae*, yield loss, damage, injury

PO065

THE EFFECTIVENESS OF BIO-PESTICIDES AGAINST RICE LEAF FOLDER (*CNAPHALOCROCIS MEDINALIS*) TO USING THE DNA QUANTIFICATION AS PESTICIDES SCREENING TOOL

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Studies were carried out to check the efficacy of different bio pesticides at equal concentration against *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae) in institute of Agriculture science university of the Punjab, Lahore. In this study DNA quantification was used as pesticide screening and evaluating tool for plant Genome health three botanicals crude extracts *Azadirachta indica*, *Melia azedarach*, *Eucalyptus globulus* and one microbial insecticide Bt were used in this experiment for control of rice leaf folder. RCBD were used with 3 replication. Results indicate that Neem crude extract gave excellent results with lowest post treated increase in leaf infestation (2.76%, 3.07% and 6.60% after 3, 9 and 16 days of application respectively) caused by *Cnaphalocrocis medinalis*. Bt showed good results with least increase in post treated infestation of 4.48%, 6.06 and 9.33 after 3, 9 and 10 days respectively after neem, *Melia azedarach* and Eucalyptus also control the Cnaphalcocrocis medinals but with least efficacy. Maximum leaf folder infestation was observed in control plots. Chemical pesticide cause genotoxicity and harmful for plants, in this experiment we applied bio pesticide and checked it may bond or not with plant genome. DNA from the rice plant treated with different bio pesticide including control plot were isolated using CTAB method and then run on gel electrophoresis after DNA confirmation DNA were Quantified with spectrophotometer. Our bio pesticide treated rice plant sample results showed DNA quantification value 34.45 µg/ml, 33.65 µg/ml, 31.05 µg/ml, 34.55 µg/ml, 33.88 for control, Bt, Eucalyptus, Darek and Neem respectively. These differences are negligible and may be due to device calibration or observation error. Hence bio pesticides are safe and cause no genotoxicity of plant genome.

Keywords: Bio-pesticides, rice leaf folder, (*Cnaphalocrocis medinalis*), DNA Quantification, pesticides.

PO066

CEREAL APHID FEEDING BEHAVIOUR – COMMONALITIES AND DIFFERENCES DEPENDING ON APHID SPECIES AND WHEAT SUSCEPTIBILITY

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Cereal aphids can cause significant problems for farmers, affecting yield directly by feeding and indirectly by spreading viruses, such as the barley yellow dwarf virus. For some aphid species, such as the Russian wheat aphid (*Diuraphis noxia*) resistant varieties are commercially available, whereas for other aphid species in cereals, control is mainly via insecticide applications. We have studied the development and feeding behaviour of three cereal aphid species, *Rhopalosiphum padi*, *Sitobion avenae* and *D. noxia* on wheat lines ranging from modern elite varieties, landraces and wild relatives with an aim to identify wheat lines with resilience to aphids. During this process we have observed variability in aphid performance and present here results showing how aphid feeding behaviour differs depending on aphid species, host-plant susceptibility and mycorrhizal association. We discuss possible explanations for these differences, the need for novel technologies for aphid assessment and implications for aphid control and management in agro-ecosystems.

Keywords: Aphid, wheat, crop protection

PO067

FIRST INSIGHTS IN EGGPLANT RESPONSE TO *FRANKLINIELLA OCCIDENTALIS* ATTACKS

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Eggplant (*Solanum melongena*) belongs to the Solanaceae family, which includes crops of economic importance. Among biotic stresses affecting solanaceous crops, western flower thrips (WFT) *Frankliniella occidentalis* causes severe economic losses worldwide, due to both its feeding and oviposition on plant tissues, and its high competence to transmit the tospovirus Tomato spotted wilt virus. Nowadays, WFT is mainly controlled by insecticides; insect-resistant crops may avoid or reduce the use of pesticides, but their development requires the identification of effective sources of resistance as well as of the involved genetic mechanisms. Therefore, a wide set of eggplant lines was used to identify more resistant and susceptible genotypes to WFT. Choice experiments were carried out by offering simultaneously the genotypes to insects, using both the whole plants as well as leaf discs (antixenosis assays). The capacity of WFT to live and develop on the genotypes was assessed in no-choice experiments using leaf discs (antibiosis assays). The identified more resistant and susceptible genotypes were used to investigate genes involved in eggplant response to WFT by transcriptome sequencing at three time points (0-12-48 hours) after insect attack for both the lines. Total RNA was extracted, stranded libraries prepared and sequenced (Illumina). For each sample, about 66 millions of paired reads were generated. After quality controls, reads were cleaned/trimmed and aligned to eggplant genome and statistics of differentially expressed genes calculated. Overall, in both lines several genes were found to be up or downregulated, suggesting their involvement in a general response of the plant to thrips attacks. On the other side, genes up and down regulated in the resistant or susceptible genotype at a specific timepoint were also detected, and based on their annotation, some of them may be involved in the response to stress and insect attack.

Keywords: western flower thrips, antibiosis assays, antixenosis assays, resistant/susceptible genotypes, *Solanum melongena*

PO068

STUDYING *IPS SEXDENTATUS* BORNER OUTBREAKS IN ITALIAN COASTAL PINE STANDS: TECHNICAL ISSUES BEHIND TRAPPING IN WARMER CLIMATES

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During the last few years the stenographer bark beetle *Ips sexdentatus* Borner has been causing serious damage to pine stands on the coast of Tuscany. Here, its populations have dramatically increased in number, exploiting the weakening of maritime pines by other pests, as well as by the increasingly frequent wildfires. Preliminary results of an ongoing multi-year study on *I. sexdentatus* population dynamics including its control in these areas have shown issues regarding the use of aggregation pheromones for monitoring and mass trapping. According to the manufacturer, dispensers should be replaced after 50 days, thus guaranteeing efficacy until the 50th day. This may be true for colder climates in inland pine stands, but the warmer summer temperatures on the coastline of southern Tuscany deplete dispensers considerably faster. By dividing the traps into two groups (A and B) which were baited about 30 days apart, we assured the presence of adequately baited traps in the field. Trapped beetles were counted in both groups every 10 days for 10 months in 2017. While spring and fall temperatures in these areas did not significantly affect dispensers' nominal life, catches decreased noticeably after 30 days during summer. More specifically, when at the 50th day one group registered almost zero catches, the other one (never more than 30 days after its own bait renewal) still recorded 15-20 catches per trap. Thus, in warmer climates, using pheromone dispensers according to manufacturer instructions may lead to errors in interpreting flight patterns and the number of *I. sexdentatus* generations per year. Thus, the study of this pest's outbreaks in newly exploited areas must reconsider the life-span of pheromone dispensers according to the local climate, both to properly interpret the flight curves and to better address control strategies.

Keywords: bark beetles, aggregation pheromones, integrated pest management

PO069

EVALUATION OF A DELTA-SHAPED TRAP FOR THE MONITORING OF OLIVE FRUIT FLY WITH PARTICULAR REFERENCE TO COLOUR, SIZE AND SELECTIVITY TOWARD NATURAL ENEMIES

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The olive fruit fly *Bactrocera oleae* (Gmelin, 1790) (Diptera: Tephritidae) is considered the most serious pest of olive in many areas throughout the word, affecting the quality and quantity of oil and table olives during the infestations in late summer and autumn. The research focused on the comparison of some commercial or modified devices, with particular reference to delta-shaped Jackson trap, baited with ammonium carbonate for the monitoring of *B. oleae*. The traps were also evaluated for their selectivity toward predator and parasitoids. Monitoring tests were conducted in three sites located in a hilly interior of southern Italy, at 300-600 m/asl. Tests lasted between the end of August and the beginning of November 2016. We compared in each test three to four devices in a Latin square experimental design. To have a standardized comparison of different size traps, in all tests except when Bottle trap was used, a correction was applied to the olive fruit fly counts, to ensure that the sticky surfaces of the traps resulted equivalent. One test evaluated the performance of Jackson trap having yellow/white sticky surface with bottle trap and yellow sticky panel. Other tests assessed the performance of Jackson traps baited or not baited with ammonium carbonate and modified using different colors and sizes of the support or the sticky surface. Without applying the correction, sticky panel captured significantly more olive flies and natural enemies, followed by Jackson trap with yellow panel, whereas Jackson trap with white panel and Bottle trap had lowest captures. The results showed that catches of olive flies in Jackson trap increased by: ammonium carbonate presence, size of device and extension of the yellow surface. In case of natural enemies, ammonium carbonate increased catches of parasitoids; size and color improved captures of both predators and parasitoids with a combined effect.

Keywords: Olive fruit fly, monitoring, ammonium carbonate, Jackson trap

PO070

REMATING IN *CERATITIS CAPITATA* STERILE MALES (WIEDEMANN) (DIPTERA: TEPHRITIDAE): A POSITIVE FACTOR IN SIT PROGRAMS

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Sterile Insect Technique (SIT) is used, among other biological control tools, as sustainable measure for the control of *Ceratitis capitata* in many agricultural regions where this pest can trigger severe economic impacts. To determine the potential on SIT and enhance its field application, the biology and ecology of *C. capitata* has been widely investigated. The tendency of wild females to remate has been deeply studied. Nevertheless the remating potential of the released sterile males remains unknown. Here, the remating capability of sterile males was quantified under laboratory conditions. Virgin females were offered to sterile males, both of the same age and nutritional state, up to four times for copulation: 24 hours, 48 hours, 4 days and 7 days after the first mating. At the end of each test, males were separated according to response, mated or unmated, and subsequently reused for the next round of mating assays. The frequency of remating in each test was obtained. In addition, the sperm in the testis of the sterile males was analyzed by direct observations under microscopy and the sperm transfer success was also estimated by quantitative Real-Time PCR on the spermatechae of mated females after each remating. Initial results suggest that sterile males are not only capable of remating 24 hours after the first copulation but also a second time at 48 hours. Indeed, sterile males have been observed to remate up to four times. Sterile sperm has been detected in mated females at each remate. These findings shed light about the mating opportunities for sterile male, an aspect until now underestimated in many SIT programs.

Keywords: Mediterranean fruit fly, irradiated males, multiple mating, spermatozoa quantitative Real-Time PCR

PO071

MONITORING AND PUPULATION GROWTH OF TOMATO RUSSET MITE, *ACULOPS LYCOPERSICI* MASSEE (ACARI: ERIOPHYIDAE) USING GREEN LABEL STICKER

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This study is conducted to develope a new method of early occurrence monitoring for *Aculops lycopersici* Massee (Acari: Eriophyidae), a major pest in tomato. *A. lycopersici* causes damage to plants such as curling of leaflet edges, followed by leaflet bronzing. As feeding continues by the mites, the plant takes on a russeted, wilted appearance, eventually culminating in leaflet desiccation and finally plant death. Fruit damages in tomato were shown when the density of mites was very high. Also, *A. lycopersici* caused more damage to unripe fruits than ripe fruits. The time taken to detect *A. lycopersici* on green label sticker was 7.0 seconds, the shortest. Blue, orange and white label stickers took 17.1, 19.8, 12.3 seconds respectively. The permeable microscpoe with side illumination was useful in observing *A. lycopersici* on label stickers. *A. lycopersici* began to occur in late-April and the density of the mites increased rapidly after mid-May. The density peaked in mid to late-June, and decreased after late-June. A lycopersici was observed on lower stems 20 days after the release of the mites and observed on higher stems 60 days after. The peaked densities were observed on stems 40 and 60 days after and on leaf 80 days after. The label sticker is enough to monitor early occurrence of *A. lycopersici* in tomato cultivation facilities.

Keywords: *Aculops lycopersici*, Russet mite, Monitoring method, Tomato

PO072

NEW FRONTIERS OF IPM: PANTHEON (PRECISION FARMING OF HAZELNUT ORCHARDS) PROJECT FOR THE IMPROVEMENT OF PEST CONTROL STRATEGIES

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We are presenting PANtHEOn (Precision Farming of Hazelnut Orchards), a project funded by the H2020-SFS-2016-2017 Call, Research and Innovation Action, Topic SFS-05-2017, which aims to develop an industrial Supervisory Control And Data Acquisition (SCADA) system to be used for the precision farming of large orchards of hazelnut (*Corylus avellana* L.). This system involves the use of a limited number of heterogeneous unmanned robotics components, *i.e.* aerial platform (UAV) and ground vehicles able to navigate in the orchard, to collect data and perform some of the most common farming operations, principally regarding the plant growth habits, yield and phytosanitary status. Particularly, it will be developed a system useful for the quantification of pest incidence in the orchard using multispectral visible and near infrared (VNIR) and thermal data collected from the UAV and the ground robots, and for optimization of the quantities and the typology of pesticides used for insect pests control. Collected field data will be sent to a central unit able to assess the plant's health and to suggest the decision-making process concerning the required agronomic interventions. The following pests will be considered: *Phytoptus avellanae*, *Curculio nucum*, and true bugs (*Halyomorpha halys*, *Gonocerus acuteangulatus*, *Palomena prasina*, *Piezodorus lituratus*, *Raphygaster nebulosa*, *Nezara viridula* and *Dolycorum baccarum*) involved in economic detriment due to the quality losses by kernel abortion, malformation and emergence of unpleasant flavor. This will result in a better average state of health of the orchard, and in an increased effectiveness of Integrated Pest Managements (IPM).

Keywords: Robotics, Crop protection, Stink bugs, Phytosanitary status

PO073

ELECTROANTENNOGRAPHIC AND BEHAVIORAL RESPONSES OF SPITTLEBUG *PHILAENUS SPUMARIUS* TO SELECTED ESSENTIAL OILS

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Philaenus spumarius (L.) (Hemiptera, Aphrophoridae), known as "meadow spittlebug", is a highly polyphagous species widespread in the Holarctic Region. Nymphs and adults are "xylem-feeders" mainly on actively growing ones. This species has been indicated as one of the vectors of *X. fastidiosa* Wells *et al.* (1987) strain subspecies pauca, involved in the "Olive Quick Decline Syndrome" in the Salento Peninsula (Southern Italy). The identification of compounds interfering in the host selection processes, could be of practical interest in control strategies towards the spittlebug to prevent sap sucking from olive plants. Moreover attractive compounds could be used in monitoring systems. Our study was carried out to evaluate the capability of the peripheral olfactory system of female and male *P. spumarius* adults to perceive volatile organic compounds (VOCs) of essential oils (EOs) extracted from *Lavandula officinalis*, *Citrus aurantium*, *Cymbopogon nardus*, *Pelargonium graveolens*, *Salvia officinalis*, *Helichrysum italicum*, *Rosmarinus officinalis* and *Thymus vulgaris*, reported as repellents to many harmful insects. Furthermore behavioural responses of *P. spumarius* to *L. officinalis* and *C. aurantium* EOs were also evaluated using Y-tube bioassays. Electroantennographic recordings (EAG) showed that male and female antennae were able to perceive the EOs, and increasing concentrations of the them elicited dose-dependent EAG responses, in spite of the low number of antennal sensory structure described in *P. spumarius*. At certain concentrations, olfactory responses showed that males were attracted by *L. officinalis* and repelled by *C. aurantium* EOs. Females were attracted by *C. aurantium* at lower concentration and repelled at higher ones. This information provides interesting indications in the perspective of EOs practical applications.
Research supported by Apulia Region, CISOL Project, DD n. 494, 2016.

Keywords: *P. spumarius*, essential oils (EOs), Electroantennographic recording (EAG), behavioral bioassay

PO074

INVESTIGATIONS ON NEW RESISTANCE SOURCES AGAINST TO SUNN PEST FROM WHEAT GENOTYPES OF ANATOLIA

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Eurygaster integriceps is still an important pest in some parts of Anatolia, the gene center of wheat. Despite many studies have been carried out on the pests of wheat, which have important effects on the quality and quantity of wheat production, no significant progress has been made. Based on the results of studies done within the last five years, a total of 135 wheat genotypes obtained from the national gene bank, ICARDA, CIMMYT and USDA were tested in controlled cages, namely having four infested and two control cages without infestation in both vegetative and generative period with *Eurygaster integriceps*. Genotypes were planted in hill plots in cages and a female and male adult sunn pest were released to each of them. Within the scope of this study, evaluations of were made in two stages, vegetative and generative. The characteristics measured in vegetative evaluations were white ear, white head and the number of spikes per plant, whereas in the generative period, the values of number of sucking symptoms, damage ratio, thousand grain weight, sedimentation and delayed sedimentation have been considered. Based on the findings in this work, sedimentation and delayed sedimentation values of six genotypes which are promising in terms of durability were measured and also, the results of the second year were found similar to the first year. The sedimentation and delayed sedimentation values with the genotypes that are mentioned as tolerant in the studies were determined with the varieties of Aldane (21-7), Ceyhan 99 (21-9), Snowmass (16-5), Sönmez (24-8) and ICARDA (line number 100) (21-6) and the landrace (line number 71) (14-3). The genotypes identified as resistant in the vegetative period in previous studies have not found sufficient resistance in this investigations that emerged from generative evaluations.

Keywords: Sunn pest, resistance, wheat genotypes, Anatolia

PO075

MATING BEHAVIOUR AND DUAL MODE COMMUNICATION OF PEAR PSYLLA *CACOPSYLLA PYRI*

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Cacopsylla pyri (L.) (Hemiptera, Psyllidae) is one of the most important pests of European pear, and its management generally depends on the use of chemical insecticides, but *C. pyri* outbreaks are sometime observed. Ecological control strategies should be desirable and the knowledge of mating behavior is crucial to develop new ones. A multi-approaches research aimed to acquire knowledges about *C. pyri* mate finding. Electroantennographic (EAG) analyses and olfactometric bioassays were used to evaluate the activity of intraspecific semiochemicals on *C. pyri*. The EAG amplitudes revealed that volatile compounds, present in female cuticular extracts, elicited dose-dependent responses in males, indicating that these compounds were able to stimulate the male olfactory system. In behavioral bioassays, living females and female cuticular extracts, attracted summerform males in a highly significant manner. Gas chromatography-mass spectrometry revealed that 13-methylheptacosane, 11,13-dimethylheptacosane, 2-methylheptacosane and 3-methylheptacosane were found in larger amounts in female extracts than in male ones, which suggests their role in male attraction. In addition, a laser vibrometer device was used to detect a male-female substrate-born vibrations pattern during pre-copulatory period. The female vibrational signal was recorded as mp3 and conveyed, in loop using a minishaker, on pear shoots with *C. pyri* virgin pairs to interfere with the mating by masking the natural communications.
Research supported by Centro Interdipartimentale per il Miglioramento e la Valorizzazione delle Risorse Biologiche Agro-Alimentari (BIOGEST-SITEIA), Reggio Emilia, Italy.

Keywords: European pear psylla, Electroantennographic (EAG) analyses, olfactometric bioassays, 13-methylheptacosane, 11,13-dimethylheptacosane, Gas chromatography-mass spectrometry

PO076

MAIZE LEAF WEEVIL (*TANYMECUS DILATICOLLIS* GYLL): SERIOUS PEST PROBLEM IN ROMANIA

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Romania has more than 2.5 million hectares as maize cultivation which represents the highest area within the EU. However, the maize monoculture is favorable for pests. *Tanymecus dilaticollis* (Coleoptera: Curculionidae) is the main pest of the maize mainly at south and south-east part of the country. The insect is harmful when maize plants are in the early vegetation stages (BBCH 10-14). Each year, around one million hectare area is attacked by this pest with different level of attack intensities. In case of high *T. dilaticollis* invasion, the maize seedlings could not survive and the farmers have to resow their fields, causing unexpected costs. Spring drought and higher temperatures conditions are even more favorable circumstances for weevils attack. Also, maize monoculture has an increasing effect on pest density associate with higher impact of attack. Recent studies from the last decades pointed out, the chemical treatment of the maize seeds with systemic insecticides was the most effective method to reduce the loss. However, the use of neonicotinoid insecticides as seed treatment of the spring crops was restricted from 2014, according to the EU directive 485/2013. Because of this resolution, no effective insecticides are available for maize seed treatment against *T. dilaticollis* in Romania. For this reason researches are facing with the challenge to find some alternative treatments instead of neonicotinoids seeds treatment which can be permanently banned in the EU after 2018. This paper presents some results about the effectiveness of the seed treatments with imidaclopird active ingredient comparative with single vegetation spraying with deltamethrin or thiacloprid active ingredients for *T. dilaticollis* control. The experiment was carried out in south-east of Romania, between 2015 and 2017. The single spraying experiment on plants of BBCH 10-14 vegetation stage did not provide enough protection against weevils attack under high pest density (4 adults/plant).

Keywords: Maize, pest, seed, chemical, control

PO077

PORTABLE ELECTRONIC NOSE TECHNOLOGY FOR DETECTING THE BROWN MARMORATED STINK BUG

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Brown marmorated stink bug *Halyomorpha halys* is a new threat for agricultural crops in Europe, and accurate and rapid diagnostic tools are needed for a successful management of this pest. In fact, for a correct timing of control measures, it is crucial to promptly assess the presence and abundance of the pest in the field. Recently, electronic nose (e-nose) technology has been developed for identifying, characterizing and recognizing odour pattern of stink bugs or stink bug-induced damage. Therefore, this study aimed at exploring the feasibility of using a commercial available e-nose, the Cyranose-320 (Smiths Detection Inc., Pasadena, CA), for identification and discrimination of *H. halys* among other bug species, and for detection of the bug-induced damage on fruit. The instrument trained to recognize specific odour patterns gave interesting results by means of Chemometric Data Analysis software. In laboratory conditions, the e-nose proved to detect and distinguish odours emitted by undisturbed and disturbed, males and females of *H. halys* and its sensitivity increased at higher odour concentrations. Moreover, by dual comparison, the e-nose discriminated the odour emitted by undisturbed or disturbed individuals of *H. halys* from those ones of the two species *Nezara viridula* and *Gonocerus acuteangulatus*. By contrast, in field conditions, the e-nose did not accurately differentiate odours from different bug species. In laboratory conditions, it was highly accurate in discriminating between damaged and undamaged hazelnuts and apples. For promising applications in the field, further research is needed to adapt the sensor system for a more robust response to the odour of *H. halys* in different environmental conditions.

Keywords: *Halyomorpha halys*, Cyranose-320, monitoring tool, fruit damage

PO078

INFESTATION RESPONSE OF CITRUS LEAFMINER, *PHYLLOCNISTIS CITRELLA* (LEPIDOPTERA: GRACILLARIIDAE) TO SYNTHETIC AND ORGANIC FERTILIZATION UNDER FIELD CONDITION

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Citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is one of the most serious pest of citrus nursery stock and cause extensive damage to young flushes. The objective of this study was to evaluate the effect of different compost (Vermicompost, BioFert, Tara Root) and NPK fertilizers on CLM activity in nursery plantations of *Citrus reticulata* Blanco. All three organic composts were applied at 0.25 and 0.5kg/plant and the synthetic fertilizer was applied at 0.2 and 0.4kg/plant to assess the effect of these fertilizers on CLM infestation. Results showed that percent CLM infestation was significantly ($P<0.001$) varied after application of compost and NPK fertilizer during Fall-2016 and Summer-2017. The control rate of CLM was high after application of BioFert compost during both seasons. The percent CLM infestation was only 0.72% during Fall-2016 and 6.03% during Summer-2017 after the application of BioFert compost at 0.5kg/plant and the infestation was comparatively low as compared to control treatment. Thus, BioFert compost might be considered as promising and potential fertilizer in the management of the leafminer population. Further research is needed to determine the factors responsible for reducing larvae population and whether these organic fertilizers can be utilized in future citrus IPM programs as a tool for citrus leafminer management.

Keywords: Compost, synthetic fertilizer, *Phyllocnistis citrella*, *Citrus reticulata*

PO079

OLFACTORY RESPONSES OF *FRANKLINIELLA OCCIDENTALIS* PERGANDE TO ATTRACTANTS OR REPELLENTS UNDER PLANT-ODOUR ENRICHED CONDITIONS

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Frankliniella occidentalis Pergande (Thysanoptera: Thripidae), the western flower thrips, is a major pest in agricultural and horticultural crops worldwide. The volatile compounds methyl isonicotinate, p-anisaldehyde, eugenol, and linalool are known as olfactory attractants, and salicylaldehyde is known as a repellent for *F. occidentalis* under clean air conditions in laboratory experiments. In the present study we assessed the responses of *F. occidentalis* to these compounds when presented alone, in combination, and in the presence of background odours emanating from various flowering or non-flowering plants such as cucumber, capsicum, chrysanthemum, clove basil or lavender plants in a Y-tube olfactometer. In the presence of any background plant odour, the attractiveness of pure methyl isonicotinate to *F. occidentalis* proved to be consistently significant. Compared to clean air conditions, a slightly lower percentage of thrips chose the Y-tube arm loaded with 10% p-anisaldehyde in the presence of cucumber leaf odour. With non-flowering clove basil plants in the background, *F. occidentalis* responses to 1% eugenol, a constituent of clove basil essential oil, were neutral, and the same applied to responses to pure linalool, a constituent of lavender essential oil, in the presence of flowering lavender plants. Also, thrips responses to pure or diluted salicylaldehyde were clearly influenced by plant background odours. We simulated a push-pull situation and found a trend indicating that the percentage of *F. occidentalis* choosing the airflow loaded with the attractant methyl isonicotinate was higher when the airflow in the other arm of the Y-tube was loaded with the repellent salicylaldehyde compared to clean air, and vice versa. We showed interactions between attractive or repellent volatile compounds and the environmental odours in the chemical ecology of *F. occidentalis* and the potential of a combined use of these compounds in thrips pest management.

Keywords: Thysanoptera, western flower thrips, push-pull, behavioural control, methyl isonicotinate, p-anisaldehyde, eugenol, linalool, salicylaldehyde, allelochemical

PO080

MATING DISRUPTION OF CODLING MOTH, *CYDIA POMONELLA* L., BY APPLICATION OF CIDETRAK® CMDA COMBO™ MESO™ DISPENSERS IN BULGARIA

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The codling moth (CM) *Cydia pomonella* (L.), is the main pest of pome fruits worldwide, including Bulgaria. Its larvae cause severe damage to apples, pears, quinces and walnuts. Resistance of CM to commonly used conventional insecticides (organophosphates and pyrethroids), which has already been noted in Bulgaria, as well as restrictions on insecticide use imposed by EU regulations, have encouraged a new approach to the control of this pest. Alternative methods have been extensively tested during the last decades. Mating disruption appears as a very promising solution. The aim of this study was to test the effectiveness of mating disruption (MD) for control of codling moth, in apple orchards comparing CIDETRAK® CMDA COMBO™ MESO™ dispensers at high and low rates of 80 and 20 dispensers per ha. The trials were carried out during the years 2016-2017 in North East of Bulgaria. Monitoring of CM flight was carried out by sex pheromone trapping in the years of the study. PHEROCON® VI Delta, sticky traps, were installed in the trial orchard using a scheme provided by the producer. In the trial orchard the traps were baited with standard PHEROCON CM L2 – codlemone as well as with PHEROCON CMDA COMBO caps and AA lures. The traps, lures and dispensers were developed and are manufactured by Trécé Inc., USA. For comparison, PHEROCON® VI Delta sticky traps were installed in a reference orchard located in the same region, which was treated with insecticides only. All pheromone traps were checked twice a week. The damage to apples was inspected during the season and at harvest on 1600 apples. Fruit damage in the trial plot was compared with that in the reference orchard, located in the vicinity, which was treated with conventional pesticides. And even in late cultivars, fruit damage by CM in the MD plot was in the frame of the economic threshold.

The usage of CIDETRAK® CMDA COMBO™ MESO™ dispensers at reduced rates of 80 and 20 dispensers per ha shows that the number of dispensers used does not affect the effectiveness of mating disruption. The reduced rate of dispensers used will help growers to decrease labor in the field. Applications of these dispensers can provide effective control of codling moth, with better results than the conventional protection programmes employed in Bulgaria.

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Keywords: IPM, mating disription, codling moth, apple, CIDETRAK® CMDA COMBO™ MESO™ dispensers

PO081

TEST OF THE INSECTICIDAL ACTIVITY OF FOUR PLANTS AGAINST BLACK BEAN APHID (*APHIS FABAE*)

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With the increase of the world's population, the demand for food has increased and accompanied by use of pesticides, which constitute a risk to the health and the environment. This work aims to study the effect of aqueous extracts of four plants (*Artemisia herba-alba*, *A. campestris*, *Myrtus communis* and *Ruta montana*), on the larvae of *Aphis fabae* under laboratory conditions, through the screening of their efficiency on the mortality of aphids. We adopted two methods of extraction: maceration and infusion. For each method, the mix of the plant powder and distilled water was filtered and then diluted to obtain 3 concentrations for each plant species. To test the insecticidal effect of plant extracts, we used in total 24 treatments with 3 repetitions for each one, in addition to the control. So, we have prepared 75 Petri dishes. Each one contained 1 treated leaflet of faba bean. We counted the number of dead larvae 3, 6, 12 and 24 hours after the artificial infestation. In addition, a phytochemical screening was carried out to detect the presence of certain secondary metabolites in the extracts studied. Results showed that the extract obtained from *A. campestris* by infusion at a concentration of 25% seemed to be more efficient with a higher mortality rate (53.33% after 24 hours of the artificial infestation) followed by *A. campestris* 15% by maceration and *M. communis* 5% by infusion (50 and 43,33% of mortality rate respectively). Regarding the phytochemical screening, we observed that the four plants have polyphenols, tannins and saponins. It is suggested that the quantity of these metabolites and their types may have a key role in the effectiveness of each extract. The use of aqueous extract of *A. campestris* may be one of the important components of IPM program against black bean aphid.

Keywords: aqueous extracts, aphids, biopesticide, faba bean, desert wormwood

PO082

INSECTICIDE SPRAY TIMING TO CONTROL THE ASIAN CORN BORER, *OSTRINIA FURNACALIS* (LEPIDOPTERA: CRAMBIDAE) IN WAXY CORN FIELDS

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Waxy corns are usually planted at late April in Korea, and those fruits were mainly used for light meals. Application timing of insecticide to control the Asian corn borer, *Ostrinia furnacalis*, which is the most serious pest against corns, was studied in the fields. Insecticide sprays at 3 and 10 days after adult peak time in a pheromone trap survey showed higher control effects, based on evaluation with leaf and stem damage, than more earlier and late sprays. An artificial inoculation of neonate larvae onto corn plant near adult peak time induced higher damage than at other times. The results indicated that the adult peak time is an important decision point for controlling of *O. furnacalis* larvae with insecticide. During the effective control time, most larvae in the field were found as stages from the first to fourth instar in the whorled leaves of corn. Larvae were located in stem, stamen, and fruits during the following season till fruit harvest. In a time-course counting of each larval instar number, densities of the third and fourth larvae again increased at fruit harvest time after the previous final minimum. It suggested that the second generation of larvae located in corn plants at that time. A nonlinear temperature-dependent development model of overwintering larvae during the period from 1 January till adult emergence was evaluated as an alternative tool for detecting the adult peak time without pheromone trap monitoring. In late-planted fields, however, single spray of insecticide near adult peak time could not reduce damages of stem and fruit, and larval density. It was postulated that the second generation of larvae is responsible for the damage. The result suggested that plant stage-dependent timing of insecticide spray is needed for several planting during a wide range of season.

Keywords: *Zea mays*, *Ostrinia furnacalis*, control, insecticide, spray time

PO083

GENETIC RESOURCES FROM NATIVE CORN FOR SOURCES OF RESISTANCE TO THE ASIAN CORN BORER, *OSTRINIA FURNACALIS* (GUENEE) IN THE PHILIPPINES

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The Asian corn borer (ACB), *Ostrinia furnacalis* (Guenee), is the most serious pest of corn in the Philippines. This pest became effectively managed when Bt corn was introduced in 2013. However, ACB remains a serious problem for open pollinated varieties (OPV). The search for alternative and safe control measure continues especially for OPVs. A banner program entitled "Corn Germplasm Utilization through Advanced Research and Development (CGUARD)" has been spearheaded since 2015 by the Department of Agriculture through its Bureau of Plant Industry and Regional Field Offices and in collaboration with the Institute of Plant Breeding, University of the Philippines Los Baños. A primary objective is to collect and conserve native corn with one of the projects targeting to search for sources of resistance to ACB. To date, at least 2,000 corn accessions have been collected and only 20% have been screened for corn borer resistance. In addition, the field performance and status of these genetic resources may be initially assessed vis-à-vis the last 15 years of Bt corn planting. This paper aims to present the significant efforts of the Department of Agriculture to collect and conserve the native corn in the country and pursue one of its ultimate goal of developing resistant ACB varieties with improved yield and responses to other biotic and abiotic factors.

Keywords: genetic resources, native corn, Asian corn borer, *Ostrinia furnacalis*, CGUARD

PO084

SUGAR AND AMINO ACID PREFERENCE IN THE ANTS *LASIUS NIGER* AND *FORMICA RUFA*

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Numerous ant species establish mutualistic relationships with hemipterans, improving the survival of honeydew-producing pests; *e.g.* aphids, and interfering with the control of said pests by natural enemies. Interestingly, disrupting this ant-aphid mutualism would not only help the biological control of aphid pests, but would also allow us to harness the beneficial effects associated with ant presence, *e.g.* soil improvement, plant protection against disease. While alternative sugar sources can disrupt this mutualism to some degree, ants do not always accept artificial sugar solutions, suggesting that a more complex formulation is needed. Thus, our work has focused on analyzing ant preference for different sugar and amino acid components. Laboratory experiments were carried out with black garden ant *Lasius niger*, and field experiments were performed with red wood ants of the *Formica rufa* group. While there seems to be a general trend to prefer (i) disaccharides over monosaccharides and (ii) solutions containing a protein component over those with only sugar, results show that ant-preference for the various constituents is highly context-dependent and that substances that can act as attractants are species-specific. Hence, while general guidelines can be provided, the ultimate formulation of a solution capable of out-competing aphid honeydew in the field should be tailored to the specific ant species.

Keywords: *Lasius niger*, *formica rufa*, ant nutrition, food preference, amino acid, sugar, ant nutrition

PO085

EFFECTS OF DIFFERENT INSECTICIDES ON WHITEFLY *B. TABACI* MEAM1 FEEDING BEHAVIOR AND ON THE TOMATO CHLOROSIS VIRUS (TOCV) TRANSMISSION IN POTATO PLANTS

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The whitefly *Bemisia tabaci* causes severe losses in potato crops, not only due to feeding damage, but mainly because it transmits important viruses, such as Tomato chlorosis virus (ToCV) (genus Crinivirus), which is transmitted semi-persistently by *B. tabaci*. Although insecticides are widely used to control *B. tabaci*, they may not be efficient to control virus transmission and this whitefly species shows resistance to a vast majority of pesticides available. In this study, we evaluated the effect of different insecticides on stylet activities of ToCV-viruliferous adults female of *B. tabaci* Middle East Asia Minor 1, on potato plants (cv. Ágata) treated with: acetamiprid, cyantraniliprole, flupyradifurone and a control with water, using the Electrical Penetration Graph (EPG) technique. The insects were placed on the potato plants 72 h after the insecticide application (systemic effect) and EPG recorded for 10 h. The results showed that on plants treated with flupyradifurone the insects remained less time in phloem activities (waveform E), performing less waveform E1 (associated with salivation into phloem sieve elements), and none of the insects in this treatment were able to perform waveform E2 (correlated with passive phloem sap uptake from sieve elements). In acetamiprid-treated plants, the insects spent less time performing the waveform E1, but the other parameters did not differ from the control. The insecticide cyantraniliprole did not differ from the control treatment in any of the evaluated parameters. Although the three insecticides are able to kill *B. tabaci* at to 72 h after application (greenhouse assay), only flupyradifurone is able to affect the feeding behavior of this vector in order to hinder the inoculation of ToCV to healthy plants, since it is already known that the inoculation of ToCV is associated with phloem acitivities (mainly waveform E1).

Keywords: Electrical Penetration Graph, Whitefly control, Chemical control, Virus-vector-relationship, Crinivirus

PO086

INFLUENCE OF *BACILLUS THURINGIENSIS* APPLICATIONS DURING DIFFERENT GRADATION PHASES ON POPULATION DYNAMICS OF *LYMANTRIA DISPAR*

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The gypsy moth, *Lymantria dispar* (L.), is the main pest of cork oak forests, causing extensive defoliation during both the progradation and the culmination phases. In order to control its infestations and protect cork oak foliage, aerial applications of *Bacillus thuringiensis* serovar kurstaki (Btk) formulations have been annually carried out in Sardinia since 2001. Our work aimed to investigate how sprayings made in different gradation phases affect both abundance and temporal patterns of the gypsy moth. Data of Btk-applications carried out on eight cork oak forest districts of Sardinia from 2005 to 2010 were analyzed to test our hypothesis. Sprayings during both progradation and culmination phases were effective in protecting the oak canopies in the same year of the treatment and reduced the pest abundance in the following year. However, Btk applications made during the culmination phase caused the progressive decrease of *L. dispar* infestations over time, whereas the population abundance increased again after 2-3 years when the spraying was applied during the progradation phase. Our results indicate that treatments applied during the progradation phase shift forward the outbreak peak rather than suppress the gradation.

Keywords: Gypsy moth, control, population abundance

PO087

ECOLOGICAL IMPLICATIONS OF PLANT RESPONSES TO BRIEF AND GENTLE TOUCH

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Mechanical stimuli are among the most common cues to which plants respond with physiological, morphological or biochemical adjustments in order to adapt their growth and enhance survival in a specific environment. Mechanical contact between plants may represent an important cue for early detection of the presence of potential competitors. Changes in plant status induced by touch may be detected by herbivory insects providing them with reliable cues about host plant quality. The broader ecological significance of induced plant responses to mechanical stimuli on pest insect behavior has not been explicitly investigated. In recent studies we examined whether plant exposure to one min daily touching over a period of 6 days may affects aphid host search behavior. To mimic contact between nearby plants, a soft face brush was used to apply light and brief mechanical stimuli on the leaf surface. Maize, bean and potato plants responded to touch by changes morphology and volatile emission. Our treatment did not cause trichome damage nor change their total number on touched terminal leaves. However, gently touching increased density of glandular trichomes and the number of pavement cells on part of leaves that have not been treated. *Rhopalosiphum padi*, *Aphis faba*, *Myzus persicae* and *Macrosiphum euphorbiae* showed significantly reduced acceptance of touched plants compared to untouched controls. Touching altered the volatile emission of treated plants that released higher quantities of specific volatile compounds that were less attracting for *Coccinella septempunctata* then those released by controls. Taken together our studies demonstrated that plant mechanical interaction may have ecological effects beyond the plant itself, affecting organisms at higher trophic levels.

Keywords: Aphids, ladybirds, volatiles, aphid host plant acceptance,

PO088

SOLANUM PANDURIFORME: A PROMOSING BOTANICAL FOR APHIDS CONTROL ON BRASSICAS

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Brassicas are important vegetable crops for home consumption and market gardening in eastern and southern Africa. Productivity is affected by aphids through their feeding and disease transmission. Botanical insecticides can potentially manage aphids, but few plants have been evaluated for use on brassicas. A study was conducted to evaluate the effectiveness of *Solanum panduriforme* to control aphids on brassicas. Extracts from three parts of *S. panduriforme* were assessed for their aphicidal effects on the cabbage aphid, *Brevicoryne brassicae*. Extracts from leaf powder (LP), ripe berry powder (BP), fresh ripe berries (RB) and fresh unripe berries (UB) were extracted with four solvents: water, ethanol, hexane and diethyl ether, using homogenisation, maceration and solvent-assisted / sequential extraction methods. Extracts effectiveness was determined using laboratory bioassays and plant assays in screen house and field conditions. Assays were designed as completely randomized with three or four replications depending on the assay. The immature (LP and UB) parts were generally more effective than the mature (BP and RB) parts; mortalities ranged from 90% down to 67% respectively. Ethanol extracts were more effective than aqueous extracts (LP 96% and 63%; BP 96% and 64%; RB 100% and 64%; UB 100% and 90%). The dried crude extracts from hexane were more effective than di-ethyl ether extracts. Group chemical analysis indicated presence of alkaloids in the berries (BP, RB, UB), and absent in the leaves (LP). Phenolic compounds and flavonoids were present in all the extracts (LP, BP, RB, and UB). Saponins were present in the fresh parts (RB, UB). The locally available *S. panduriforme* plants can be used as an aphicide to control aphids on brassicas. Farmers can directly prepare an easy and cheap botanical insecticide from leaf powder and unripe berries using water. Ripe berries can be investigated further for development of a commercial botanical insecticide.

Keywords: Aphicide, bioassays, brassica, *Brevicoryne brassicae*, effectiveness, extracts, mortality, *Solanum panduriforme*

PO089

TOMATO PROSYSTEMIN: STRUCTURAL AND FUNCTIONAL CHARACTERIZATION

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Systemin (Sys) is a 18-amino-acid peptide hormone which, upon insect attack, is released from its precursor protein, Prosystemin (Prosys) to initiate a complex signaling cascade that leads to the production of defense compounds (Ryan, 2000). The tomato genome contains only one copy of the *Prosys gene*; it is composed of 4176 bp and is structured into 11 exons, of which the last one codes for Sys. Sys peptide was traditionally considered as the principal actor of the resistance towards pests observed in tomato plant overexpressing the ProSys. However, recent evidences (Corrado *et al.*, 2016) suggest that ProSys N-terminal region could contribute to defense response. This observation prompts us to investigate the biochemical and structural features of the Prosys protein. To this purpose Prosys cDNA was amplified, cloned into pETM11 vector and expressed in BL21 (DE3) *E. coli* strain. The recombinant protein was purified by three chromatographic steps: Immobilized Metal Affinity Chromatography, Ion Exchange Chromatography and Size Exclusion Chromatography. After each step of purification, protein purity was assessed on 15% SDS-PAGE. Since the beginning Prosys showed peculiar behavior (Délano *et al.*, 1999) as observed by SDS-PAGE and chromatographic approches. Moreover, bioinformatics and structure prediction tools allowed us to evaluate the intrinsic features of the protein and to analyze the secondary and tertiary structure of the prohormone. The results show, for the first time, that Prosys is an Intrinsically Disordered Protein (IDP) (Buonanno *et al.*, 2017). Finally, plant assays revealed that the recombinant pro-hormone is biologically active being very effective in the induction of tomato defence-related genes, which confer protection against *S. littoralis* larvae both locally and sistemically (Buonanno *et al.*, 2017). To our knowledge, this is the first biotic stress related IDP identified in plants. Studies aimed at a deeper characterization of ProSys function are presently in progress.

Keywords: Tomato, plant defense, precursor, IDP, structural and functional characterization.

PO090

EFFECTS OF THE LEAF GALLING DENSITY OF *GRAPE PHYLLOXERA* ON SANGIOVESE GRAPE PRODUCTIONS

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Daktulosphaira vitifoliae Fitch, the *grape phylloxera*, has represented the main problem for *Vitis vinifera* since its introduction in Europe, at the end of the 19th century, until French scientists proposed to use native American Vitis spp. as rootstocks to control this pest. Grafted, resistant

grapevines have solved the issue for decades, nevertheless, in the 80's leaf galling infestations appeared in Italy becoming more common and serious recently. Up to now, few studies have been carried out on the effects of leaf galling generations on grapevine growth and production. Since severe grapevine leaf infestations were recorded in Tuscany, a study was conducted in two vineyards in the Siena province in order to evaluate possible effects on grape production. The phylloxera infestation was monitored by sampling Sangiovese vines (*V. vinifera*) in summer 2017. Infested plants were divided into 3 different groups according to the infestation level and compared to uninfested vines. Grapes were sampled and addressed to technological and phenolic analyses. Although the weather has been very dry for the entire experiment, it has been possible to evidence a remarkable effect of the leaf infestation on grape quality. The phylloxera leaf galling incidence decreased as the temperatures increased. The photosynthetic rate and gas exchange decreased as the infestation level increased. Technological and phenolic analyses carried out on grapes highlighted a reduction of the grape quality. As a matter of fact, grapes from heavy infested vines showed reduced sugar and polyphenolic contents. These preliminary findings confirm the necessity to carry on further researches aimed at investigating the phylloxera population dynamics in order to define appropriate economic thresholds for applying control methods against this pest.

Keywords: *Grape phylloxera*, leaf galling infestation, Tuscany, photosynthetic rate, grape quality

PO091

DAMAGE ASSESSMENT OF SUNN PEST *EURYGASTER INTEGRICEPS*

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Agro-ecosystems of wheat and barley consist of biotic and abiotic factors which influence the crop production directly and indirectly. They are perpetually modified by mankind to increase the yield. Therefore, clear understanding of the mentioned factors and their interactions would be very important in quality and quantity conservation of an ecosystem fauna. Among the insect species in wheat and barley, Sunn pest, *Eurygaster integriceps* Put. (Het: Scutelleridae) is the most destructive biotic agent. The pest is an ancient problem in Iran and many countries in Pale-arctic region which damages to the crop quantitatively (feeding on leaves and stems by adults which results in partial or complete spiels whitening and low grain production) and qualitatively (direct feeding on grains by nymphs and decreasing baking property of flour made of the damaged grains). Therefore, wheat as a strategic crop is covered by insurance to ascertain farmers regarding the crop loss compensation. However, it has not been easy to estimate the amount of yield reduction in damaged fields especially in case of quality measurement. Having said that, a study was carried out in order to obtain a guideline for Sunn pest damage and crop loss assessment to be used by insurance experts. The EIL was considered a base for quantity and quality damage to the crop. According to the results, the quantity damage was determined as 332.8, 294.4, 255.4, 226.4 and 227.2 Kg/ha in Pishtaz, Mahdavi, Shahriyar, Zarin and Alvand wheat varieties, respectively. Similarly, the quality damage in the same varieties was measured as 20520000, 14130000, 7200000, 46620000 and 20520000 Ril/ha, respectively.

Keywords: Sunn pest, Wheat, Quantity and Quality damage

PO092

MANAGEMENT STRATEGIES TO REDUCE WIREWORM DAMAGE IN POTATOES

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The larvae of the click beetles (Coleoptera: Elateridae), wireworms, are identified as a major pest of potatoes and other vegetable crops worldwide. Over the past ten years, a steady increase in the population of an invasive species *Agriotes sputator* in Prince Edward Island (PEI), Canada, resulted in its spread into un-infested crop land. The use of pheromones was the main approach employed to trap adults, but mass trapping of male beetles to reduce the population has been unsuccessful. Our objective was to create a trap to attract both male and female beetles that could be used to reduce the population in an infested field. In 2015, a light trap (NELT™) was invented and tested. The trap consists of a solar powered spotlight, a 16oz glass as a pitfall trap, and a ¼ inch wire cage to prevent by-catch of larger insects such as carabid beetles. The traps were placed in two wireworm infested fields in PEI and collected every two days from May 14 – July 30. Collected beetles were counted and sexed. Results showed significantly higher numbers of female and male beetles in the light traps compared to the control. A total of 930 beetles (40% females and 60% males) were collected per trap. Further studies in 2016 and 2017 confirmed the NELT to be an efficient method to remove females from the population prior to egg laying. Evaluation of a mass trapping strategy coupled with crop rotation using buckwheat or brown mustard, which has been shown to reduce tuber damage by 85%, and the use of an insecticide during the potato phase is underway. Preliminary results show a reduction in wireworm population in infested fields using this strategy.

Keywords: Wireworms, NELT, trapping, crop rotation, potato, management

PO093

A NEW CHANCE IN THE CHESTNUT ORCHARD IPM: IDENTIFICATION OF ECOTYPES AND CHARACTERIZATION OF THEIR RESISTANCE TO *DRYOCOSMUS KURIPHILUS*

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The Asian Chestnut Gall Wasp (ACGW), *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) is considered one of the most impactful invasive pest of *Castanea sativa* Mill. The management of ACGW is challenging and the control obtained through the introduction of the parasitoid *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) needs a variable number of years to reach satisfying levels. The use of resistant cultivars could be an efficient and eco-friendly choice for implementing the parasitoid use. A recent study, carried out in Southern Italy, showed the presence of an ecotype expressing resistance against ACGW. Starting from the results of this study our activities were extended to several different ecotypes. Ecotypes were characterized through morphologic and genetic methodologies taking into account also the commercial aspects. The second step was the characterization of resistance carried out by observing ACGW preimaginal stages and the respective effects on trees. The percentage of bud infestation, the number of eggs into the buds, the number of larvae inside the buds and galls, the healthy leaves

and shoots, and the number, size, and position of galls were evaluated. Ecotypes were able to counteract the development of ACGW acting at different levels. Results showed a reduction both of the laid eggs and surviving larvae resulting in a decreased number of galls on the branches. Usually, a progressive mortality of cynipid larvae was recorded. Sometimes it culminated with the total larval mortality at budburst. Because a resistance phenomenon expressed against an organism could affect other pathogenic organisms bringing out a cross-resistance, the ACGW resistant trees were also tested against the causal agent of nut rot in chestnut, *Gnomoniopsis castaneae* Tamietti (syn. *G. smithogilvyi*), whose infestation in the last years has aroused particular concerns for chestnut orchards. Preliminary results showed the endophytic presence of G. castaneae in almost all the resistant tested trees.

Keywords: ACWG, ecotype, galls, Gnomoniopsis, Torymus

PO094

S.I.M.FITO AND S.I.M.FITO-LAB: NEW TOOLS TO IMPROVE THE MANAGEMENT OF PHYTOSANITARY EMERGENCIES

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The increasing phytosanitary risks due to the development of international trades asked for the development of rapid strategies useful to prevention, management and long-term control of emergent pests. In the last years the development and dissemination of Geographical Information Systems (GIS), has given a big boost to the monitoring of agro-forestry environments and green areas, especially through the opportunity to associate quantitative data to geographic representations. The Plant Health Service of the Campania Region, in collaboration with Ariespace, conceived and developed two software applications “SIMFito” and “SIMFito-Lab” whose principal aim is an accurate and prompt archiving of the phytosanitary emergences and of laboratory test results. SIMFito (Informative System for the Phytosanitary Monitoring) is a web application, directly interconnected with the Eppo Global Database, that firstly allows the online filling of the data-sheets resulted from the monitoring and inspection actions. Thus the geographic database is updated in real-time and can provide information both on the host-plants and the pest organisms, either as table or as map format. SIMFito allows to manage and analyse large amounts of data thus allowing the study of spatio-temporal variations of pests populations and their effects on the economy and plant health. SIMFito-Lab efficiently completes the collection of information in the fields, managing and archiving the laboratory analyses conducted on collected samples, allowing the quick production of reports and laboratory certificates. S.I.M.Fito and S.I.M.Fito-Lab were conceived to facilitate both the data collection in the field/laboratory and their management in accordance with national and community commitments. Moreover, the monitoring information in real-time allows a rapid delimitation of the infested and buffer zones, promoting a prompt planning of the attempts of eradication and/or containment of emergent pests.

Keywords: Eppo database, GIS, monitoring, quarantine pests, software

PO095

THE RESISTANCE OF DIFFERENT VARIETIES OF *FABA BEAN* (*VICIA FABA* L.) TO FEEDING BY THE BLACK BEAN APHID, *APHIS FABAE* SCOPOLI

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The *faba bean*, *Vicia faba* L. (Fabaceae), an important crop well adapted to most climatic regions of the world, is widely used for food and livestock feed, and to increase nitrogen fixation in the soil. The black bean aphid, *Aphis fabae* Scopoli (Homoptera: Aphididae), which can be a serious pest of *faba bean*, infests different varieties to a different extent. Therefore, the present study was performed to assess the resistance of different varieties to *A. fabae*. A total of 16 varieties, namely three registered varieties, Seher, Eresen and Lara, and 13 local varieties, were collected from different regions of Turkey. The present study was carried out as field experiments in 2016 and 2017 in Samsun, Turkey. A randomized complete block design with three replications was used. An infestation scale rating from 0 to 3 was employed to classify the size of the aphid populations on the individual varieties. Counts were done during a two week period in May. The percentage of the plants of each variety that were infested by the aphid was evaluated with the Townsend-Heuberger formula. Analysis of variance (ANOVA) and the t-test were used to compare the infestation level of the varieties, and means of the first and second countings, respectively. There were no significant differences among the varieties regarding the infestation level; G13 and G3 had the highest infestation level (57.5%) and lowest infestation level (24.4%), respectively. There was a significant difference between the first and second countings of the infestations ($P<0.05$). The mean infestation rate increased during the two week period from 31.18% to 35.99% but infestation rates did decrease in some varieties, namely Seher, G3, G6, G9, G10.

Keywords: *Aphis fabae*, black bean aphid, *faba bean*, resistance, Samsun

PO096

ANALYSIS OF CROATIAN *CYDIA POMONELLA* L. (LEPIDOPTERA: TORTRICIDAE) POPULATION VARIABILITY BY USING GEOMETRIC MORPHOMETRICS

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Geometric morphometrics was used to analyze codling moth population variability in integrated and ecological systems of apple production in Croatia. The aim of study was to investigate the connection between different pest management types and *C. pomonella* morphology. Altogether, ten field populations (six from integrated and four from ecological apple production) and one laboratory grown population, which has never been exposed to insecticides, were sampled. The wing morphology of 294 *C. pomonella* were investigated using standard geometric morphometric procedures based on the forewing venation of 18 landmarks. Data analysis was divided into two groups: 1) field populations *versus* laboratory population; and 2) integrated *versus* ecological populations. The results demonstrated a morphological difference in field populations *versus* laboratory population and integrated *versus* ecological populations. This study presents an affordable and accessible technique that reliably demonstrates wing shape and size differences which can be used to reveal populations differences and thus can be used as a biomarker. Overall implications from this work suggest that geometric morphometric techniques can be used to detect population changes related to different type of apple production and could serve as alternative ‘biomarker’ to more expensive and specialized-use genetic markers, when investigating biological adjustments.

Keywords: wing morphology, pest mamagement types, apple production, codling moth

PO096a

IMPORTANCE OF CITRUS NON-COMMERCIAL AREAS AS D. CITRI AND HLB INOCULUM SOURCE

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The importance of primary spread and the need of a regional management among neighbouring commercial properties is already known for HLB, but almost no study has emphasised the importance of non-commercial citrus areas as inoculum sources. Our objective was to evaluate the influences of commercial and non-commercial areas, conducted with poor or no HLB management, to *D. citri* detection and HLB incidences in a property under strict HLB management located in São Paulo State. Data was obtained regionally from five neighboring areas: Reference, P1, P3 (commercial areas), P2 and Residential (non-commercial areas). Reference area performed frequent inspection/eradication of symptomatic trees and detection/chemical control of *D. citri*. P1 and P3 performed *D. citri* chemical control, only. P2 and Residential areas, despite presenting citrus HLB symptomatic trees and *D. citri*, didn’t perform HLB management. P1 and P3 were localized on West side, next to Reference area and P2 and Residential areas were localized on East side (±2.0 and 1.7 km, respectively). Traps were placed, from December 2012 to December 2016, for detection of *D. citri* in the five areas. Additional data of *D. citri* detection and trees eradication was provided by Reference area. *D. citri* detection and HLB incidence and AUDPC were higher on East side of Reference area. The average numbers of *D. citri* adults captured in traps was 0.022 and 0.004 on East and West sides of Reference area, respectively. P1 and P3 presented 0.039 and 0.005 averages of adult per trap, respectively. The non-commercial P2 and Residential areas presented 0.182, 0.239 (time 1) and 1.769 (time 2) *D. citri* adults per trap, respectively. Non-commercial areas without HLB management and at longer distances appeared to have a greater effect on *D. citri* detection and HLB incidence than neighboring commercial areas that performed some chemical control of *D. citri*.

PO097

BIOLOGICAL INSECT PEST MANAGEMENT PROTOCOL FOR CABBAGE CROP

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Indiscriminate use of insecticides results in resistance development in insects and high maximum residue levels (MRL) in vegetables. Diamondback moth (DBM), *Plutella xylostella* L., among insect pests is the most destructive pest in cabbage. The availability of parasitoids locally, their mass rearing technique and field release techniques were investigated to develop a protocol for the management of DBM using parasitoids and the control of other insect pests using botanical insecticides was the objective of the present study. Experiments were conducted in the laboratory and farmers fields in three locations; Marassana, Thalathuoya and Nuwara Eliya, Sri Lanka. Locally available larval parasitoids *Cotesia plutellae* was used in the experiments. Thirty five low cost artificial diets formulated by replacing expensive ingredients of standard Beiver and Boldt diet with locally available ingredients was investigated to mass rear the parasitoid in the laboratory. Three diets gave comparative results to fresh cabbage leaves based on mean life span, mean pupal weight, sex ratio and fecundity of DBM. Field experiments in five farmers’ fields each were carried out on cabbage in Nuwara Eliya, Marassana and Thalathuoya locations revealed a release rate of 1500 parasitoids/ha were the best without augmentation releases. The highest parasitism obtained in locations Marassana, Nuwara Eliya and Thalathuoya was 100%, 66% and 80% respectively. Introducing parasitoids 2-3 weeks after transplanting of cabbage gave significantly higher parasitism. Cost of cultivation of cabbage with the developed protocol for insect management was compared with the farmer practice of cultivating indicated a saving of Rs.100, 355.00 (699.03 USD @ Rs.150.00/USD)/ha/crop. Results concluded a biological pest management protocol where 1500 parasitoids/ha of *C. plutellae* to be introduced to the field at 2-3 weeks after transplanting to control DBM and neem seed kernel (24 kg/ha) water extract for the control of other insect pests.

Keywords: *Plutella xylostella* L., biological pest management, parasitoid, *Cotesia plutellae*, Cabbage

PO098

A PORTABLE EXTRACTION DEVICE FOR ASSESSING POPULATIONS OF CROP PESTS AND THEIR NATURAL ENEMIES

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A hand-held extraction device has been developed for assessing numbers of small arthropod pests and their natural enemies on field-collected samples of leaves, flowers or fruit. The cylindrical device was constructed by modifying and joining two “Lock & Lock” plastic tubs with “Clip Seal” removable lids, with a disc of 3 mm aluminium “Mod Mesh” forming a sieve partition at the junction between the tubs. The top lid is removed and plant samples are placed onto the upper sieve surface, next to a newly-opened vial containing a cotton wick wetted with methyl isobutyl ketone (MIK). The top lid is then closed to create a seal, allowing the MIK to act as a fumigant for a 20-minute exposure period, anaesthetising any arthropods present. The base can then be removed, allowing small arthropods that have fallen from the plant material, through the sieve and onto the plastic to be counted and identified using a hand lens or microscope. Calibration experiments were carried out under laboratory conditions by inoculating strawberry button fruit with known numbers of the predatory mite *Neoseiulus cucumeris*, at a range of densities. Linear regression analysis revealed a very close correlation between numbers of mites recovered using the extraction technique and the numbers on the fruit surface, suggesting that the device provides a reliable assessment of numbers of arthropods present on the plant material sampled. Under field conditions, the device has been deployed to sample populations of western flower thrips (*Frankliniella occidentalis*) and its predators including *N. cucumeris* and *Orius* sp. in commercial strawberry crops. The method is particularly useful for sampling very small arthropods such as *N. cucumeris*: the device has revealed the presence of these beneficials on flowers and fruits, even when they cannot be detected by examining plant surfaces using a hand lens.

Keywords: Population sampling, predatory mites, thrips, natural enemies, strawberries

PO099

INVESTIGATIONS ON THE INSECTS ASSOCIATED WITH QUINOA (*CHENOPODIUM QUINOA*) IN NORTHERN ITALY

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Quinoa (*Chenopodium quinoa* Willd.) is a chenopodiaceous plant native to South America, cultivated for centuries mainly in Bolivia, Peru and Ecuador, countries which together account for more than 80% of world production. This herbaceous plant has wide potential for use: in the human diet (both in grain form and as flour and derivatives), as animal feed, and also in non-food and industrial applications, although the main interest is the former, due to its nutritional qualities and because it is gluten free, not being a cereal. In recent years, cultivation has been undertaken in several European countries, including Italy, where quinoa is grown both in experimental plots and in open fields. The study of the insects found on this crop has been carried out above all in South America but also in the United States and, more recently, in some European countries, where experimental cultivation began several years ago. In Italy, where the spontaneous and closely related *Chenopodium album* L. is present, it is particularly interesting to investigate which insects adapt to feed on the new crop. It is very likely that quinoa attracts indigenous-weed insect pests. As part of the experimental agronomic tests carried out recently to verify the adaptability of quinoa to the territory of Piacenza and Pavia (northern Italy), surveys have been carried out on the insect pests of this plant. In 2016, three test fields of quinoa and one second-level variety comparison field were sampled, while in 2017 the sampling was carried out in four second-level variety comparison fields. The results obtained show that the crop is attacked in the early vegetative phases by aphids (*Rhynchota Aphidoidea*) and flea beetles (*Coleoptera Chrysomelidae*), while in the flowering phase until ripening the most harmful insects are bugs (*Rhynchota Pentatomidae*, *Coreidae*, *Miridae*). Attacks by flea beetles, if combined with particularly dry periods, can seriously compromise the crop.

Keywords: Quinoa, northern Italy, insect pests, aphids, flea beetles, bugs

PO100

COMPARISON OF DIFFERENT OUTPUTS FROM SIMULATION PROCESSES TO DESCRIBE *ANTHONOMUS EUGENII* (CANO) LIFECYCLE: AN OVERVIEW FOR VARIOUS DEVELOPMENT-RATE FUNCTIONS

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Anthonomus eugenii (Cano) (Coleoptera:Curculionidae) is one of the main pests for sweet and hot varieties of pepper, Capsicum spp. widely diffused principally in Central America. The first Italian report (2013) was in Lazio region: in the next years, the Regional Phytosanitary Service activated an eradication program that has not allowed the pepper cultivation. This invasion has aroused interests of IPM's scientists. Modeling plays a key role to reduce chemical treatments, but for a good forecast are needed adjusted tools, a good knowledge regarding the insect's biology and his typical environment. This approach is not new in IPM, and it is applied to manage many pests in different crops, for example in grapewine. The potential of one good forecast is to have a general picture of what will happen in the next days, and then evaluate following actions. The main goal of this work is to provide a series of best fit parameters related to different type of developmental rate-function, specific for *A. eugenii*, through the use of various tools built using ROOT's libraries. Actually, there is a wide choice of shapes to describe experimental points, but we want to analyze which is the most appropriate for our goals. We simulated *A. eugenii*'s lifecycle with Distrubuted-Delay Model at different constant temperatures (17, 19, 21, 24, 27, 29, 31, 32°C), only varying the development rate function in input: we compared the linear, the Logan, and Briére rate-functions built with data from Toapanta (2005). We focused on differences between mean developmental times, above all near the higher and lower threshold for the insect's life. Results showed that the best representing functions are Logan and Briére, which have no significant differences in outputs. The linear rate function become unreliable as the temperature rises.

Keywords: Distributed Delay Model, ROOT, Integrated Pest Management, Pepper cultivation

PO101

ATTACHMENT ABILITY OF THE GREEN STINK BUG *NEZARA VIRIDULA* (HETEROPTERA: PENTATOMIDAE) ON ARTIFICIAL SUBSTRATES AND HOST PLANT SURFACES

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The present investigation characterizes the attachment ability of males and females of *Nezara viridula* L. (Heteroptera: Pentatomidae), a polyphagous insect representing a cosmopolitan pest, on artificial surfaces (smooth hydrophilic, smooth hydrophobic, different surface roughness) using a centrifugal force tester and a traction force experiments set ups. Moreover, the effect of different host plant species characterized by smooth, hairy and waxy surfaces on the bug attachment ability is evaluated through friction force experiments. Surfaces of different tested plants and attachment devices of bugs are studied in Cryo-Scanning Electron Microscope. Potential damage to insect attachment devices induced by walking on different leaf surfaces is evaluated. In case of the plant *Phaseolus vulgaris*, where insects showed a strong reduction in their adhesion ability after walking on the leaf, the damage to the insect by two cultivars with different morphological features and the insect ability to recover after 24 h is revealed. Strong attachment forces produced by bugs on various surfaces, characterized by different morphological features, together with bugs' ability to recover after the damage to attachment devices is in agreement with the great adaptability and ecological plasticity of this widely-spread bug species. The present study, increasing our knowledge on the mechanical interaction of this species with different host plant species, can potentially contribute to development of new strategies to control this insect pest.

Keywords: Friction, adhesion, pulvilli, hairy pads, claws, insect-plant interaction, trichomes, wax crystalloids

PO102

DEVELOPMENT AND REPRODUCTION OF *GRAPHOLITA MOLESTA* AND *GRAPHOLITA DIMORPHA* (LEPIDOPTERA: TORTRICIDAE) ON IMMATURE FRUITS OF PEACH, PLUM, AND APPLE

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Both *Grapholita molesta* (Busck) and *Grapholita dimorpha* Komai are important pests of pome and stone fruits. Mature fruits of peach and plum have been tested as food sources for *G. molesta*. But, no studies have ever been conducted on immature fruits that are available more likely for spring population of *G. molesta* and *G. dimorpha*. Thus, we evaluated immature fruits of peach, plum, and apple as food sources by assessing their biological and behavioral attributes in laboratory. Those immature fruits were collected on May-June in 2016 and 2017. The 1st instar larvae of *G. molesta* preferred peach the most with the exiting rate of 81.0% which was lowest among the fruits tested. In *G. dimorpha*, plum fruit was preferred the most with lowest boring rate (20.0%) and exiting rate (50.0%) on peach fruit. Larval period of *G. molesta* was also shortest in peach fruit (11.8 d) while that of *G. dimorpha* was shortest in plum fruit (10.9 d). However, *G. molesta* laid more eggs when reared on plum fruit (132.6) than on peach fruit (67.0). *Grapholita dimorpha* laid no egg on peach fruit. In conclusion, immature fruits of both plum and apple are better food sources for both *G. molesta* and *G. dimorpha* than immature peach fruit.

Keywords: oriental fruit moth, fruit diet, development, fecundity

PO103

CYDNIGPRO – DEVELOPMENT OF A DECISION SUPPORT SYSTEM TO REGULATE PEA MOTHS *CYDIA NIGRICANA*

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The pea moth *Cydia nigricana* can cause severe damages in commercial pea production (*Pisum sativum*). The larvae develop inside pea pods and feed on grains. To prevent damages on peas and to apply the least needed amount of insecticides the Central Institute for Decision Support Systems in Crop Protection (ZEPP) develops a new decision support system (DSS). The phenological part of the DSS predicts a site specific coincidence of pea plant flowering and the pea moth's main flight, representing the highest risk for an infestation (oviposition) of the pea pods. The resulting coincidence indicates the need of insecticide applications. In order to obtain precise spaying dates, population dynamics allow the calculation of a critical date when L1 hatch is starting. Therefore, it is essential to simulate pea plant ontogenesis and pea moth development. The simulation of pea ontogenesis (SIMONTO) is based on rated BBCH-stages, temperatures from meteorological stations, and day length in three model areas within Germany. It is developed for dry and green peas with early and late sowing dates. For acquiring data concerning the development of the respective pea moths stages (pupae, moth, egg, larvae), trials in climate chambers and greenhouses were carried out at different temperatures and started with hibernated cocoons. Additionally, data from field studies, such as flight activity and larval appearance over time, were collected to calibrate the model. The site specific DSS will be available on the website of ISIP e. V. (www.isip.de) for farmers and consultants.

Keywords: *Cydia nigricana*, *Pisum sativum*, ontogenetic model

PO104

DEFINITION AND POPULATION DYNAMICS OF "GREEN LEAFHOPPERS", A COMPLEX OF SYMPATRIC PESTS SPECIES IN ISRAELI VINEYARDS

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In Israel, the leafhopper (Homoptera: Cicadellidae, Typhlocybinae) pests constitute a complex of species which is commonly referred to as "green leafhoppers". They are polyphagous, causing damage to vineyards and many other crops. The species are phenotypically similar and therefore are monitored as a complex and not at the genus or species level. These sympatric leafhoppers may compete on the same niche or occupy separate niches in time of activity or host plant preference. First we identify the species in the complex by analyzing the reproductive organ in a sample of males, collected from vineyards. We found three defined species: *Asymmetrasca decedens*, *Jacobiasca lybica* and *Zygina rhamni*. We then developed species-specific primers for each of the three species to distinguish among the sympatric species without being solely dependent on males. To test the activity of each species in the field, we monthly collected leafhoppers, by vacuum, from adjoining plots of two vine cultivars- Cabernet sauvignon and Merlot. The specimens were counted, separated by gender and identified by the species-specific primers. We found that *A. decedens* was the dominant species in the spring in both cultivars and *Z. rhamni* was dominant later in the season with some overlapping between the two species. However, time of appearance of *Z. rhamni* differs between cultivars. The 3rd species, *J. lybica* also appears at mid-summer but is more sporadic and its population is lower. We currently study the developmental rate of each species on the two cultivars and the effects of the possible competition on their population size.
Acknowledgement: We would like to thank Dr. Valerio Mazzoni (Fondazione Edmund Mach) for his help in morphological identification of the species.

Keywords: Leafhopper, Sympatric species, Population dynamic

PO105

IDENTIFYING PRE AND POST-ALIGHTING RESISTANCE TO *RHOPALOSIPHUM PADI* (BIRD CHERRY-OAT APHID) IN ANCESTRAL WHEAT LINES

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There are approximately 4,000 aphid species, the majority of which are phloem feeders and damage their host plant by extracting nutrients during feeding, excretion of honeydew which attracts saprophytic fungi reducing the photosynthetic ability of the plant, and lastly, the transmission of viruses. *Rhopalosiphum padi* (L.) (Bird cherry-oat aphid) is a pest of many Gramineae including wheat which is a stable crop of societies worldwide. Currently,

insecticides are the main method used to control crop aphid populations, however, with increased aphid resistance to insecticides and restrictions on insecticidal classes, effective aphid population control must look towards integrated pest management strategies. There are currently no modern wheat varieties with resistance to *R. padi*. Ancestral *Triticum monococcum* (L.) lines have been shown to possess some antibiosis (post–alighting) resistance to *R. padi*, but it is unknown whether this resistance is present at different growth stages. Aphid development, survival and reproduction were investigated on one, two, 12 and 20 week old *T. monococcum* lines differing in resistance to *R. padi*. It is unknown if there is antixenosis (pre-alighting) resistance in *T. monococcum*. Volatile organic compounds (VOC's) were extracted from non-infested and aphid infested plants using air entrainment technique. Aphid behaviour towards these VOC's were determined by olfactometry. Resistance observed in the laboratory was explored in field conditions, monitoring aphid populations (*R. padi*, *Sitobion avenae*, *Metopolophium dirhodum*) and the presence of natural enemies (Coccinellidae, Crysopidae larvae, parasitized aphid). Results showed that the presence and degree of post-alighting resistance was dependant on both the resistant genotype and plant age. Pre-alighting resistance was dependant on the resistant genotype and the presence of infestation, indicating that aphids can distinguish between more and less suitable hosts. Field observations revealed that the presence of both aphids and natural enemies was determined by host plant susceptibility to aphids.

Keywords: Aphid, antibiosis, antixenosis, *Rhopalosiphum padi*, temporal, *Triticum monococcum*, volatiles, wheat

PO106

SYMBIOTIC CONTROL OF THE OLIVE FRUIT FLY, *BACTROCERA OLEAE*

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The olive fruit fly *Bactrocera oleae* (Rossi) (OLF) is a major pest, which causes severe yield losses and quality decay of olive oil. The widespread use of chemical insecticides to control this pest is a major concern for the resulting environmental impact and food safety issues. Therefore, the development of sustainable control strategies is highly desirable. The primary endosymbiotic bacterium of the OLF, "*Candidatus Erwinia dacicola*", is essential for successful larval development in unripe olive fruits. Then, targeting this endosymbiont with antimicrobial compounds may exert a control action against OLF. Here we evaluate the impact on OLF endosymbiont of Copper Oxychloride (CO) and the fungal metabolites Viridiol and Harzianic Acid (HA) produced by two biocontrol strains of *Trichoderma* spp. Laboratory bioassays were carried out on OLF wild populations to assess the effect of the oral administration of these compounds on mortality and fecundity of adult flies, and on larval development of their progeny in unripe olive fruits. Treated females were processed by qPCR to measure the endosymbiont load in the oesophageal bulb and in the midgut. Exposure to Viridiol and HA had a strong negative impact on endosymbiont load and OLF larval survival, while CO negatively affected both adults and larval stages, showing a combined toxic action and an anti-symbiotic effect, which was dose-dependent. These results provide new insights on the symbiotic control of the OLF and pave the way for developing new strategies based on the use of natural compounds with antimicrobial activity.

Keywords: *Trichoderma*, IPM, real-time PCR, antimicrobial activity, Copper

PO107

FACTORS AFFECTING WOOD ANT DISTRIBUTION IN THE BIAŁOWIEŻA FOREST

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Wood ants (the species of the subgenus *Formica* s. str.) are common in coniferous, mixed and deciduous forests of the Palaearctic ecozone, where they are one of the most important group of insects due to their high abundance and activity. The Białowieża Forest is one of the largest and best preserved forest ecosystems within the temperate zone in Europe, and is widely regarded as the best example of an ancient forest with limited anthropogenic impact, in terms of observations and research into pristine deciduous and mixed forests. We conducted a survey of wood ant mounds in an area of 1400 ha in the Białowieża Forest (N-E Poland) and related physical mound characteristics to the surrounding forest properties. We recorded the following ant mound parameters: diameter at the base, mound height, exposure of the longest slope and the distance to the closest tree (only for active mounds). In addition, we estimated the illumination condition (well-lit, moderate shade and full shade). The overall density of inhabited wood ant mounds was 0.13 ha-1. *Formica polyctena* and *F. rufa* were the most abundant species, and the highest densities of their mounds were found in fresh mixed deciduous and fresh mixed coniferous forests. The physical mound properties, like direction of the longest mound slope and the distance to the closest tree did not differ significantly between these two ant species. A significant factor affecting mound size and closest tree distance was the light condition, and consequently mound diameter, height and volume increased with increasing shade. The distance to the closest tree was greater under well-lit conditions than under shaded conditions. The results of our study suggest that coniferous forests with an admixture of deciduous trees are the most promising for maintaining vital wood ant populations in the forest.

Keywords: Wood ant, *Formica* polyctena, *Formica rufa*, Białowieża Forest

PO108

MANAGEMENT STRATEGIES TO CONTROL THE CAPE GRAPEVINE LEAFMINER, *HOLOCACISTA CAPENSIS*, IN SOUTH AFRICA

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A native leafminer, *Holocacista capensis* Nieukerken & Geertsema (Lepidoptera: Heliozelidae), was detected in a table grape vineyard in the Western Cape province, South Africa (2012), soon after stricter Maximum Residue Levels (MRL) were imposed. Since 2012, considerable effort has been exerted to gain clarity on the taxonomy of the moth; develop an attractant; establish action thresholds associated with bunch infestation; and explore the moths' bioecology and distribution throughout the Western Cape. The aim of this study was to contribute to an integrated pest management strategy, focused on the effective control of the leafminer. Various commercially available products (predominantly chemical control agents) and a biological control agent, namely entomopathogenic nematodes (EPNs), were tested against the leafmining larvae of H. capensis, collected locally. The trials were conducted by means of exposing 20 to 30 leafmining larvae to each of the chemical and biological treatments. Larval mortality was recorded after 48 h. The results indicate that certain chemicals and EPN species can effectively control larval abundance. Field studies conducted in the future will be aided by these findings.

Keywords: leafminer, Heliozelidae, table grapes, integrated pest management strategy, entomopathogenic nematodes, chemical treatments

PO109

Poster withdrawn.

Impacts of Climate Change on Agricultural and Forest Pests

PO110

DISTRIBUTION OF *BEMISIA TABACI* CRYPTIC SPECIES (HEMIPTERA: ALEYRODIDAE) IN ITALIAN HORTICULTURAL AREAS ENDANGERED BY THE NEWLY-INTRODUCED TOMATO LEAF CURL NEW DELHI VIRUS

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Bemisia tabaci (Hemiptera: Aleyrodidae) is a key pest of horticultural and ornamental crops worldwide. Within the *B. tabaci* complex, the Mediterranean (MED) and Middle East–Asia Minor 1 (MEAM1) species (formerly referred to as biotype Q and B respectively) are the most widespread in the Mediterranean basin. In the last two decades, MED populations progressively increased in those areas where intensive farming is applied. In Italy, *B. tabaci* has been recently reported to threaten cucurbit crops as vector of the newly-introduced begomovirus Tomato leaf curl New Delhi virus (ToLCNDV). Since 2015, ToLCNDV epidemics occurred in Southern and Central Italy as well as in the main islands Sicily and Sardinia, soliciting extensive surveys of the distribution of *B. tabaci* species in the affected areas. In Sardinia, whiteflies were collected on a wide range of crops and weeds at both coastal and inner horticultural sites endangered by the virus. Only MED populations were found, suggesting that the agro-climatic conditions occurring in the island have been favorable to the complete displacement of MEAM1. ToLCNDV-associated MED populations of *B. tabaci* were found to be established in Southern Lazio (Central Italy) as well, in an area where begomovirus infections had never occurred before 2016 and vector presence was thought to be only occasional. Relevant infestations of *B. tabaci* were found also in the virus-free cultivations of Northern Lazio, alerting for a possible northward spread of ToLCNDV infections. Only MEAM1 was present in this part of the region, where the lower temperatures and less-intensive farming systems probably still limit MED invasion. Further surveys of the spreading pattern of *B. tabaci* cryptic species in Italy are ongoing, to assess the evolution of the pest expansion range under the agro-ecological changes occurring in the Mediterranean basin as a consequence of the global climate warming.

Keywords: Whitefly, MEAM1 species, MED species, Cucurbitaceae

PO111

DOES THE SILK CLIPPING BY WESTERN CORN ROOTWORM (*DIABROTICA VIRGIFERA VIRGIFERA* LECONTE) ADULTS LEAD TO YIELD AND QUALITY LOSS IN SWEET MAIZE: RESULTS OF TWO YEARS FIELD STUDY FROM HUNGARY

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The western corn rootworm (WCR, *Diabrotica virgifera virgifera* LeConte) is an economic pest of maize in the USA and in most maize growing regions in Europe. Although the primary damage is caused by its larvae feeding on the root system of maize, yield losses in inbred lines and commercial grain maize due to silk clipping by WCR adults and related economic thresholds are known from Hungary. However similar data for sweet maize is not available. We aimed at determining silk feeding damage and subsequent loss in this crop. Study was conducted in field sweet maize (cv. Suregold) in Hungary in 2016 and 2017. Bridal veil cages were placed over the ear at R1 stage of maize with 0, 1, 2, 4 and 8 beetles in 2016, with 0, 1, 2, 4, 8 and 12 beetles in 2017 inside while uncovered plants were marked as untreated ones. Silk length was measured daily till the end of silking. Then adults were removed from the ear cages keeping the isolators on the ears until harvest, protecting them from other pests. After harvest, cob weight was measured and the fertility ratio assessed. Our two years results suggest that WCR adult infestation level up to 8 adults/ear is not likely to lead to economic damage, although meteorological conditions (rain, air humidity) and multiple pest presence (cotton bollworm and WCR) as well as IPM considerations (reduction of egg-laying female density) may impact the control decisions.

Keywords: Western corn rootworm; adults; silk feeding; sweet maize; damage threshold

PO112

COMPARISON OF BIO-ECOLOGY OF MEDITERRANEAN FRUIT FLY POPULATIONS AT DIFFERENT ELEVATIONS

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In this study, population fluctuations and the overwintering biology of the Mediterranean Fruit Fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), were monitored at different altitudes in mandarin, plum, peach orchards in Mersin province (Turkey). In 2014 & 2015, the effect of temperature on the development timing of immature stages of fruit fly populations were measured between the range of 18-30°C in flies collected from 200 m and 1600 m above sea level. Population fluctuation of *C. capitata* changed with altering altitude. First flight occurred at the end of April 200 m above sea level (Hamzabeyli district), while in Aslanköy (1500m above sea level) first flight occurred in mid-July. Although the fly can overwinter at 900 m above sea level (Borçak village), no overwintering was observed above 900m, and no migration was detected from sea level to high altitude plateau (1200 -1500m). The observed population dynamics via pheromone traps clearly showed five peak points of the pest population at 600 m in Haçgediği and 900 m in Borçak district, while only three were observed at 1200 m in Hangediği and at 1500 m in Aslanköy. Accumulated degree-day of *C. capitata* was calculated, based on 14.36°C developmental threshold for Aslanköy population, as 1124 degree- days, which provides enough opportunity to complete five generations

based on the thermal constant of the Aslanköy population (227.27 degree-days, egg to adult). The total developmental time of two populations which is obtained from 200 m and 1600 m showed no differences in any stage or any temperature except egg-larvae development at 22°C (student t-test).

Keywords: *Ceratitis capitata*, Altitude, highland population, overwintering, development time, Degree-day

PO113

SPECIES' RANGE SHIFTS ACROSS LATITUDES IN CHANGING CLIMATE

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Climate change is predicted to facilitate polewards shifts of species geographic ranges and thereby shape native biodiversity, ecosystem functions and services. Ecologists have primarily focused on increasing temperature, propagule pressure (*i.e.* the number of individuals and invasion events), the characteristics of an invading species and its new abiotic and biotic environment to predict the likelihood of range expansion. In contrast, by using deciduous trees, birch (*Betula pendula* and *Betula pubescens* ssp. czerepanovii) and rowan (*Sorbus aucuparia*), and birch-feeding moth species (*Epirrita autumnata*) and possible invasion of two new moth species (*Operophtera brumata* and *Agriopis aurantiaria*) and rust fungus (*Melampsoridium betulinum*) I propose that predicting range expansions across latitudes requires a more comprehensive understanding of genetic background of the species, and how species use day length to coordinate seasonal growth, reproduction, physiology and synchronization of life cycles with interacting individuals and species. Our results show that light provides selection pressure in range shifts of birch and rowan, but the response is species dependent. Furthermore, the variation in host plant quality appears not to limit the spread of the moths. However, genetic correlations in plant resistance in nature with diverse community of herbivores and pathogens may constrain birch populations from reaching optimal species-specific resistance. Finally I will emphasize that although individual species are likely to be able to adapt to the new environment, we may expect species invasions across latitudes to increase the mistiming of phenologies in communities because photoperiodism and other environmental cues may have different importance in the timing of seasonal activities in invading species compared with native species.

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Keywords: Climate change, range shifts, photoperiodism

PO114

TEMPERATURE-RELATED VOLTINISM AND DIAPAUSE IN THE WEB-SPINNING SPRUCE SAWFLY *CEPHALCIA ABIETIS*

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Cephalcia abietis is a primary pest of Norway spruce with irregular outbreak cycles. The larvae feed exclusively on spruce needles. In late summer or early autumn, the fully-grown larvae drop to the ground and construct an earth-walled chamber for hibernating. The insect spends most of its life cycle as prepupa in the soil, first as eonymph then as pronymph. Depending on soil temperatures, eonymphs develop into pronymphs in the autumn and overwinter; in the following spring they pupate and metamorphose into adult wasps. Eonymphs often remain dormant for one or several years ('extended diapause'). The variability in life cycle lengths depends both on the temperatures during larval feeding in the crown and the temperatures during nymph development in the soil. In the present study we investigated the multi-annual development of a *C. abietis* population following an outbreak in 2013 in Lower Austria (Waldviertel). We monitored the emergence of adult wasps, trapped the fully-grown larvae that dropped to the ground and evaluated the development status of the nymphs in the soil. Air and soil temperatures were recorded throughout the study period. The insects overwintered twice as eonymphs; in autumn 2015 the majority of the eonymphs had turned into pronymphs that hibernated once more. Adult wasps emerged in spring 2016. Thus, the life cycle of the population lasted for three years. Based on the lower thermal thresholds for oviposition and larval development (13°C) in the crown and for nymph (13°C) and pupation development (3°C) in the soil, we calculated that a two-degree increase in air temperatures and a one-degree increase in soil temperatures would suffice to shorten the life cycle from three to two years. A faster development time eventually reduces insect mortality during dormancy and results in higher population densities with higher and more frequent defoliation of the host trees.

Keywords: Voltinism; diapause; *Cephalcia abietis*; spruce web-spinning sawfly; climate change

PO115

EFFECTS OF PHENOLOGICAL DESYNCHRONIZATION ON SAWFLY POPULATION DYNAMICS UNDER A CHANGING CLIMATE

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With climate warming, the phenologies of interacting organisms in seasonally cold environments may advance at different rates, leading to mismatched phenological synchrony with important ecological consequences. For temperate species, the timing of early spring development plays a key role in plant-insect interactions and can influence insect performance, outbreak dynamics, and plant damage. For tenthredinid spruce sawflies, a tight synchrony of spruce budbreak and adult swarming is crucial for host colonization. Depending on the sawfly species, females oviposit on the developing needles in only just opened buds (*e.g. Pristiphora abietina*) or accept 3-5 cm elongated shoots (*e.g. Pachynematus montanus*). In this study, we recorded the spring phenologies of spruce budbreak and sawfly swarming for consecutive years (2012-2017) on lowland (450 m) and upland sites (800 m) of the Alpine foothills that were frequently infested in the past by *P. abietina* (lowlands) and *P. montanus* (uplands). The latest outbreaks in the lowland areas were exclusively caused by *P. montanus*, while *P. abietina* was no longer abundant. No damage was observed from upland sites. Since the new outbreaks occurred simultaneously at the former infestation sites of *P. abietina* climatic effects seem highly probable. Despite considerable year-to-year fluctuations in the timings of budbreak and adult sawfly emergence, tree phenologies were considerably more advanced than wasp phenologies. Specifically in spring 2014, the onset of budburst was four weeks ahead compared to a two week advanced emergence of the sawflies. While then the expanding shoots were still suitable for oviposition of *P. montanus*, they were not for *P. abietina*. We speculate that the recent shifts in the population dynamics of the two sawfly species in lowland areas resulted from a mismatch in the timing of budburst and sawfly swarming. *P. montanus* outcompeted *P. abietina*, presumably due to a lack of suitable oviposition sites for the latter.

Keywords: Climate change, phenology, budbreak, population dynamics, spruce sawfly

PO116

STARVATION RESISTANCE AND SURVIVAL TIMES OF NEONATE GYPSY MOTH, *LYMANTRIA DISPAR*, LARVAE

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Early-instar caterpillars experience very high and often very variable mortality. One key factor is phenological asynchrony with the host plant that can influence population growth or outbreak size and duration. Changing climates may alter the phenological relationships with potentially dramatic consequences for insect populations. First-instar larvae are susceptible to starvation if they do not encounter suitable food within a short time after hatching. Here we tested the starvation resistance of neonate, unfed gypsy moth larvae under different temperature and humidity conditions, both in the laboratory and in the field. Egg masses were collected in March 2017 from a forest in Burgenland (Austria) and kept at 4°C for one month. In April batches of 50 fertile eggs were incubated in climate chambers under long-day photoperiods (16L:8D) at constant 15, 20 and 25°C, respectively, with humidity levels adjusted with saturated salt solutions to either 54 or 85% r.h. The outdoor experiments started in late April, early and mid-May, respectively, corresponding to the temporal window of oak bud breaks observed in the forest. Under laboratory conditions, hatching was observed after 8-11 (15°C), 4-6 (20°C), and 3-5 (25°C) days. Under field conditions, hatching occurred after 3-10 days, depending on the air temperatures. Fifty percent of the larvae kept at 15, 20 and 25°C survived 11, 7 and 5 days, respectively. Under field conditions the larvae had a mean survival time of 7, 12 and 14 days, depending on the air temperatures. Maximum survival times of individual larvae were 18 days (15°C, indoor) and 21 days (mid-May, outdoor). Humidity levels had no significant influence on hatching and survival times. Our results show that spring temperatures can impact early-instar mortality with phenologically unsynchronized hosts. High temperatures after hatching will decrease, low temperatures will increase the temporal window of gypsy moth larvae to find suitable host leaves.

Keywords: Climate change, phenological asynchrony, starvation resistance, gypsy moth

PO117

PLANT-HERBIVORE INTERACTIONS IN THE CONTEXT OF CLIMATE CHANGE: EFFECTS OF ELEVATED ATMOSPHERIC CO_2 CONCENTRATIONS ON GRAPEVINE AND EUROPEAN GRAPEVINE MOTH (*LOBESIA BOTRANA*)

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The rising atmospheric carbon dioxide (CO_2) concentration is expected to influence interactions between plants and herbivorous insects. On the one hand, it may change the nutritional quality of the host plant and, on the other hand, affect insect phenology or biology. The European grapevine moth *Lobesia botrana* is considered as the key insect pest in European vineyards with increasing abundance worldwide. In order to anticipate future pest development and host plant response, we assessed the effects of elevated CO_2 on both *L. botrana* and grapevine, *Vitis vinifera* cv. Riesling, using climate chambers (400 and 700 ppm) and a free-air carbon dioxide enrichment (FACE; 395 and 460 ppm) system. In climate chambers, larval development of *L. botrana* was shorter and pupae were heavier under elevated compared to ambient CO_2 concentration, while the total lifespan as well as number of eggs or larvae were not affected. Whole transcriptome analysis of grapevine leaves showed that of the 14,763 genes identified, a substantial number was significantly differentially expressed during *L. botrana* herbivory. This is true for both sampling dates, fruit development and ripe berries, although the response was stronger regarding developing fruits. Altogether, more genes were differentially regulated during herbivory under elevated compared to ambient CO_2, indicating that the transcriptomic response of grapevine to *L. botrana* herbivory is CO_2-dependent. Among the respective genes, some are known to play a role in the biological processes “defense response” and “response to biotic stimuli” while the pathway of “biosynthesis of secondary metabolites” was significantly enriched. These changes are of particular interest for understanding future grapevine responses to herbivory under elevated CO_2. These results contribute to a better understanding of future grapevine-insect interactions in the context of climate change and, thus, help to identify potential consequences for future pest management strategies.

Keywords: Elevated atmospheric CO_2, FACE, plant-insect interaction, climate change, transcriptome analysis, *Vitis vinifera*, *Lobesia botrana*

PO118

PHENOLOGICAL RESPONSES IN A SYCAMORE-APHID-PARASITOID SYSTEM AND CONSEQUENCES FOR APHID POPULATION DYNAMICS: A 20 YEAR CASE STUDY

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Species interactions have a temporal component driven by environmental cues. Previous work suggests that the impacts of climate change vary between trophic levels and there is concern that this may drive shifts in community dynamics. However, the mechanisms driving phenological shifts are largely unexplored, with few long-term studies assessing mismatches of interacting species in the same spatial area – particularly for insects. Here, we use a 20-year data series on a tri-trophic system of: sycamore (*Acer pseudoplatanus*), two associated aphid species *Drepanosiphum platanoidis* and *Periphyllus testudinaceus*, and attack of these aphids by hymneopteran parasitoids. We assess climatic drivers of the phenology of all three trophic levels and the consequences of resultant trophic mismatch and direct weather effects on local scale aphid population dynamics. We do so using a moving-window approach to assess the effects of temperature and rainfall, whilst controlling for winter temperature. Warmer spring temperatures between mid-March and June were associated with advanced spring phenology of all four focal taxa, although there is inter-specific variation in the precise time period during which weather is most closely associated with phenology. Notably, we find some evidence that warmer temperatures during February delayed emergence of both aphid species, but not other trophic levels. There is thus considerable potential for asymmetrical rates of warming at different times of year to generate divergence in the phenology of interacting species. Despite this we find no evidence that aphid population growth rates were adversely impacted by the magnitude of mismatch with interacting species, or by the direct effects of climatic drivers at any time during the year. Density dependence effects were strong and thus currently appear to be capable of buffering population growth of these woodland aphid species from the marked inter-annual climatic variation that occurred during this long-term study.

Keywords: Climate change, Tri-trophic, Interactions, Phenology, Aphids

Session 2.
Behaviour

Mechanisms of Learning and Memory

PO119

MEASURING INSECT BEHAVIOR WITH THE HIGH-THROUGHPUT INDIVIDUAL T-MAZE: THE EFFECT OF SUBOPTIMAL LEARNING EXPERIENCES ON MEMORY RETENTION IN PARASITIC WASPS

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Darwinian fitness is influenced by the changes in foraging behavior through learning form continuous experiences. Parasitic wasps are ideal models to study the links between learning, foraging behavior and fitness, since they need to find suitable hosts for reproduction in complex natural environments. By learning to associate environmental cues (*e.g.* odors, colors) with rewarding experiences, such as host oviposition, or unrewarding experiences, such as encounters with non-hosts, they can improve their foraging efficiency. We investigate the effects of these sequential experiences on foraging behavior through diverse methods where parasitic wasps are exposed to increasing levels of environmental complexity, ranging from small scale T-maze set ups, to wind-tunnels and larger, semi field situations. Here, we describe the design of a new set-up for a high-throughput T-maze set-up for individual tracking of conditioned behavior, in order to do optimize efficient data collection. Groups of 36 wasps can simultaneously, yet individually, be tested in 36 individual T-maze arenas fitted in a multilayer Perspex plate system as small as 18x18cm. This system is combined with video and tracking software to automate recording of individual behavior and provides output consisting of a wide range of behavioral parameters. This bioassay is labor-efficient, due to its standardized high-throughput design, and can implemented with ease through the use of accessible techniques for manufacturing and commercially available software. The bioassay proved to be effective for measuring memory retention in two different species of parasitic wasps, *Cotesia glomerata* and *Nasonia vitripennis* and has provided us with some interesting results on the effects of suboptimal learning experiences on memory retention.

Keywords: Memory retention, parasitic wasps, *Cotesia glomerata*, *Nasonia vitripennis*, tracking system, ecology, behavior

PO120

COMPARATIVE EFFICACY OF OVITRAP COLOUR AND PATTERN TO ATTRACT MOSQUITOES IN DIFFERENT LOCALITIES OF LAHORE

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Present study was carried to find out the comparative efficacy of ovitraps having different contrasting colors and patterns, baited with grass infusion against Aedes, Culex and Anopheles gravid female vectors, during March-April 2015, at five different localities of Lahore, Punjab-Pakistan. Each experiment had 4 replicatess and the collected data were pooled for number of eggs laid in different ovitraps. Overall maximum eggs were found for Culex (21070) followed by Aedes (12120) and then Anopheles (11280) in traps. Results were found significant ($p < 0.05$) for black-red, black, red, black-green and yellow-red ovitraps by comparing with control (white) ovitraps from all collected areas, Kot lakh pat was found to be the highest positive location for pitching ovitraps. General trend observed for trap colour and pattern were same for three genera's of mosquitoes Aedes(Ae.), Culex(Cx.) and Anopheles(An.) was as follows: Black-red >black >black-green >red >yellow-red >green >black-white >yellow >black-yellow >green-red >green-yellow >white-red >white-green >white-yellow >white. Field evaluations of lethal (Boric acid, Temephos, Deltamethrin, Bti, IGR) and non-lethal (Hay and water) ovitraps baited in 10% sugar solution against Aedes, Culex and Anopheles vectors was also evaluated. Maximum eggs of Culex (6760) followed by Aedes (3970) and then Anopheles (2880) were observed. Test results were not significant ($p > 0.05$) for mean number of eggs compared to their respective control (water) and less number of eggs collected in lethal ovitraps mainly in IGR, indicating the most effective tool for controlling mosquito populations under natural conditions.

Keywords:: Mosquitoes, IGR, Colored baited ovitraps, Lahore, control

PO120a

TEMPORAL PATTERN LEARNING IN BEES

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Processing and extracting temporal regularities is necessary to make sense of stimuli available in the world, from communicative stimuli to causal relations. Abilities to process abstract regularities have been observed across different species of mammals (Endress, Carden, Versace, & Hauser, 2010; Murphy, Mondragón, & Murphy, 2008) and birds (Spierings & ten Cate, 2016; Versace, Regolin, & Vallortigara, 2006; Versace, Spierings, Caffini, ten Cate, & Vallortigara, 2017) but it is not clear whether these abilities are present in other taxa. Open questions are what abilities of temporal pattern learning are available to insects and how these abilities are connected to the communication habits of a species. Bees are ideal candidates to address this issue because only some species exchange complex information with conspecifics through the honeybee dance (*i.e.* honeybees), and because it is known that bees can master sophisticate learning tasks, including learning abstract concepts such as “same” and “different” (Giurfa, Zhang, Jenett, Menzel, & Srinivasan, 2001). Little is known, though, on their capacities to extract patterns from serially presented stimuli and whether species that do not have the waggle dance have similar pattern learning abilities to honeybees. Here we investigate the capacities of the honeybee *Apis mellifera* and the bumblebee *Bombus terrestris* to extract temporal patterns by discriminating between lights presented with different rhythms. We trained foragers to gain a sucrose reward by choosing a flashing *vs* a static light and documented bees' capacity to solve this discrimination task.

Keywords: Bees, *Apis mellifera*, *Bombus terrestris*, learning, pattern learning, temporal regularities

Communication: From Signal Production to Perception

PO121

UNVEILING THE VIBRATIONAL COMMUNICATION AND THE MATING BEHAVIOR OF THE MEADOW SPITTLEBUG *PHILAENUS SPUMARIUS* (HEMIPTERA:APHROPHORIDAE)

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The vibrational communication of *Philaenus spumarius*, insect vector of *Xylella fastidiosa*, was investigated to identify the vibrational signals associated with the mating behavior. Individuals (males and females arranged in singles, pairs or groups) were placed on a plant and the emitted signals were recorded with a laser vibrometer from the surface of a leaf. The male repertoire consisted in three types of vibrational signals. (M1) The Male Calling Song is a train of short harmonics (approximately 0.007 s) emitted either in presence or in absence of conspecifics; (M2) The Male Courtship Song is an alternation of harmonics and pulses produced either during the duet with the female or to compete with a rival during the courtship; (M3) The Male Rivalry Signal, a harmonic element of about 0.14 s, was emitted only after physical contact between males. The female repertoire consisted also of three types of vibrational signals. (F1) The Female Calling Song, made of a train of harmonics of approximately 0.008 s; the Female Response Signal, a single harmonic element emitted in reply to M2; The Female Refractory Signal, a longer harmonic element of approximately 0.14 s recorded in association with a refractory behavior (*i.e.* rejection of an approaching male). Interestingly, females became receptive to mating from September, much later than males that started to call in May. Only males that established a vibrational duet with a receptive female and maintained it throughout the searching phase eventually accomplished mating. In conclusion, in *P. spumarius* vibrational signals play an important role in the pair formation process, ensuring reciprocal identification and female localization by males. Further experiments with playbacks are required to definitely assess the specific function of the signals and also to identify candidate signals to be used for manipulating *P. spumarius* mating behavior to be used in IPM programs.

Keywords: *Philaenus spumarius*, vibrational communication, mating behavior

PO122

THE NEUROPEPTIDOME OF *SPODOPTERA EXIGUA* AND ITS REGULATION AFTER VIRAL INFECTION

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Neuropeptides are small signalling molecules produced as precursors in neurons and in neurocrine cells in multicellular organisms. These precursors are processed and modified, becoming mature peptides that will bind to their membrane receptors, regulating different aspects of the animal physiology and behaviour. *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) is a serious worldwide polyphagous pest. It is an excellent model for studying neuropeptidergic systems, contributing to the understanding of the adaptation of insects to different environments and providing new targets for the development of novel insecticidal agents. To study the neuropeptidome of *S. exigua*, RNA-seq data obtained from different tissues and developmental stages from *S. exigua* was mined for unigenes coding for hypothetical neuropeptide precursors. In total, 64 of such unigenes were identified and described based on their tissue specificity and regulation in response to different biotic and abiotic perturbations. In addition, the expression of the neuropeptidome was studied in brains of larvae infected with the *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV), an entomopathogenic virus used for the control of this pest.

Keywords: Neuropeptides, baculovirus, RNA-seq, expression changes, annotation

PO123

EFFECT OF PREDATORY BUG, *ORIUS LAEVIGATUS* SIZE ON *APHIS GOSSYPII* DEFENSIVE MECHANISMS

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Insects like other animals utilize various methods to protect themselves from natural enemies. Insect's defensive methods might differ against two kinds of predators. In this study, antipredator behaviors of cotton aphid (*Aphis gossypii*) in two stages, second and 4th instars, was determined in the presence of third instars and adult females of predatory bugs (*Orius laevigatus*). Droplet production, kicking, moving, and run away from feeding site, were determined by observation method. Study on dropped off the plant was carried out in the greenhouse condition. The results showed that the second instars of aphids just produce droplet in the presence of female adults of predatory bugs which are so aggressive predator, whereas in the presence of third instars of predatory bug they utilize another mechanism (kicking, moving and run away from feeding site). It seems that droplet production is costly and cotton aphids could grade mechanisms to save energy. Therefore, 4th instars in the presence of predatory bugs use another mechanism to save energy for reproductive. The result of this study showed that aphid could grade those defensive mechanisms. Antipredator behaviors gradation might be helpful to save energy for cotton aphids.

Keywords: *Aphis gossypii*, *Orius laevigatus*, antipredator behavior, droplet production, defensive mechanisms

PO124

ADULT-LARVA VIBRATIONAL COMMUNICATION: PLAYBACK EXPERIMENTS IN THE PAPER WASP *POLISTES DOMINULA*

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Communication through substrate-borne vibration is widespread among social insects and regulates fundamental aspects of social life. Females of paper wasp, *Polistes dominula*, by performing an abdominal oscillatory behavior known as abdominal wagging, are able to produce vibrations which propagate through the nest. Because it is widely recognized that abdominal wagging is strictly associated with the presence of larvae in the comb, it has been suggested that abdominal wagging might represent an adult-brood vibrational signal. Indeed, substrate-borne vibrations would have short-term effects related to food and trophallactic exchanges between adult and larvae. According to this, two opposite hypotheses have been proposed: a) vibrations could prepare larvae to receive food by decreasing the amount of salivary secretion, or b) they could be used by adult to stimulate the release of nutrient larval saliva. Here, we used an electro-magnetic shaker to play back the *P. dominula* vibrations on nests containing larvae. We assessed, for the first time, the short-term effect of abdominal wagging on larval behavior by recording larval response and by measuring the amount of saliva released immediately after abdominal wagging playback. Our results show that larvae a) are able to perceive the substrate-borne vibrations and b) react to abdominal wagging by increasing the movement of their body, likely to attract the attention of adult females during feeding inspection. Nevertheless, vibrations neither increase nor decrease the release of larval saliva. Although our results support the alleged role of vibrations in adult–larvae communications, they do not support the hypothesis about salivary release modulation.

Keywords: Substrate-borne vibrations, abdominal wagging, adult-brood communication, larval saliva, larval behavior

PO125

IDENTIFICATION OF VOCS FROM SUBSTRATES COLONIZED BY BLACK SOLDIER FLY LARVAE

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The molecular perception of volatile organic compounds (VOCs), linked to the processes of feeding, mating and escape from predators, is related to molecules belonging to chemoreceptive gene families, namely Odorant Binding Proteins (OBPs), Olfactory Receptors (ORs), Ionotropic Receptors (IRs), Chemosensory Proteins (CSPs). Larval black soldier flies, *Hermetia illucens* L. (Diptera: Stratiomyidae), are able to decompose large amounts of different organic materials and waste products. Larvae have a highly sensitive chemoreceptive system, specialized in perceiving molecules linked to the decomposition of organic waste. In order to test different food waste in relation to the emission of different volatile organic compounds, larvae were fed on six different substrates: 1) apple, 2) banana, 3) spent grain deriving from brewery waste, 4) apple and banana mixed in 1:1 ratio, 5) apple and spent grain mixed in 1:1 ratio, 6) banana and spent grain mixed in 1:1 ratio. VOCs sampling, through a Closed Loop Stripping Analysis (CLSA) method, was conducted two times on each substrate: at the beginning of the larval feeding process (ti) and at the end of the larval feeding process (tf). The end of the process was determined when, following daily measures, no increase or a decrease in larval weight could be registered. A VOC trap containing Hayesep Q porous polymer was connected to a vacuum pump and placed in the container with diet and larvae. Volatiles were collected for 1h at 1 l/min flow rate. At the end of the process, VOC traps were removed and the content was eluted with dichlorometane (DCM) in GC vials. The identification of volatile organic compounds (VOCs) emitted by different substrates colonized with black soldier fly larvae, linked to the functional characterization of proteins involved in chemoreception, will allow the selection of the most attractive compounds useful to study strategies to increase insect fitness.

Keywords: *Hermetia illucens*, VOCs, odorant binding proteins, fitness

PO126

HOW DOES NEST STRUCTURE SHAPE COLLECTIVE BEHAVIOR OF ANT SOCIETIES?

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Structures built by animal societies serve two main purposes: providing a shelter and offering a space for food exchanges and information transfer between nestmates. Despite the obvious influence of spatial proximity on interactions, the impact of nest structure is rarely considered in studies on social organization and collective behavior in insect societies. We compared, in the red ant *Myrmica rubra*, three nest topologies that differed by the network of galleries connecting nest chambers and thus leading to variable connectivity, centrality and meshedness of these topologies. We found that a central and highly connected chamber acting as a hub, promotes a homogeneous occupancy of all nest chambers. By contrast, a serial configuration of successive chambers makes more variable their occupancy, namely depending on their distance to nest entrance. Regardless of the nest topology, both the workers and the queen are mainly located in the entrance chamber, with a marked preference shown by the queen for the most crowded chambers. Finally, we investigated how nest topology influences two important collective behavior, which are the management of waste items and the exploitation of food resources. This namely confirmed that ant density in entrance chamber strongly influences the dynamics of food exploitation. Our study is a first step to uncover general principles on how the structure of a living space can shape the collective behavior of its inhabitants.

Keywords: Nest structure, social organization, ants, network

Reproductive Strategies of Entomophagous Insects

PO127

RELATEDNESS AND REPRODUCTIVE SUCCESS IN A QUASI-SOCIAL PARASITOID

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Sociality in the parasitoid Hymenoptera is relatively little explored, chiefly because most species are socially solitary. Several taxa of parasitoids do, however, exhibit degrees of sociality, such as maternal care via brood-guarding by single mothers (sub-sociality) or a larger number of females reproducing on the same host, in some cases providing mutual care for each other's offspring (quasi-sociality). Quasi-sociality in the Bethylid genus *Sclerodermus* takes the form of groups of females (foundresses) suppressing a host and then tending large broods of offspring until and beyond their pupation, without apparent foundress-foundress conflict over the allocation of reproduction or care. Such quasi-sociality in *Sclerodermus* has been explained in terms of direct fitness benefits to cooperation accrued to individual females that attack large hosts, as single females cannot successfully suppress and reproduce on hosts in larger size classes. Explanations of cooperative social behaviour in the Hymenoptera most often involve the consideration of inclusive fitness (kin selection) based on genetic relatedness, and kin selection may also play a role in *Sclerodermus* reproductive behaviour. Here we evaluate whether relatedness between *Sclerodermus brevicornis* females influences their success in attacking and reproducing on hosts. We vary both foundress number (2 or 4) and the genetic relatedness between foundresses (siblings or non-siblings) in a factorial design experiment, allowing us to assess numberxrelatedness interactions. We explore effects on host suppression success, and the subsequent development and sex ratios of any offspring produced. We consider the results in terms of the evolution of sociality in the Hymenoptera and in terms of the potential for *S. brevicornis*, native to Italy, to be mass-reared and released as biocontrol agents against invasive cerambycid pests.

Keywords: Sociality, *Sclerodermus*, behaviour, biological control

PO128

SPERM LIMITATION AFFECTS SEX ALLOCATION IN A PARASITOID WASP *NASONIA VITRIPENNIS*

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Insect reproduction is influenced by various external factors including temperature, a well studied constraint. We investigated to what extent different levels of sperm limitation of males exposed to different heat-stresses (34°C & 36°C) affect females' offspring production and sex allocation in *Nasonia vitripennis*. In this haplodiploid parasitoid wasp attacking different species of pest flies, we investigated the effect of the quantity of sperm females received and stored in their spermatheca on their sperm use decisions, hence sex allocation, over successive ovipositions. In particular, we compared the sex allocation of females presenting three levels of sperm limitation (*i.e.* mated with control, 34°C heat-stressed or 36°C heat-stressed males) on each host they parasitized. To disentangle the potential reduction of sperm quality after a heat stress exposure from that of sperm quantity, we also explored the clutch size and sex-ratio produced by females that were partially sperm limited after copulating with multiply mated males. Independently of their sperm numbers, all types of females produced a similar total number of offspring, but the more limited ones had fewer daughters. Sperm limitation further affected the distribution of daughters' production across time. In addition to constraints acting on female physiology, male fertility should therefore be considered in studies measuring reproductive outputs of insects submitted to heat stresses.

Keywords: Heat stress, multiple mating, sex ratio, sperm use, sperm stock, offspring production dynamic.

PO129

DIFFERENTIAL DEFENSIVE BEHAVIOR OF A DIMORPHIC APHID (*ACYRTHOSIPHON PISUM*): EFFECT OF COLOR AND SIZE

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Pea aphid, *Acyrthosiphon pisum* (Harris) (Hem.: Aphididae), consists of two color morphs, red and green, with different attractiveness to predators and parasitoids. Despite a handful of studies concerning differences of these two morphs, a quantitative study on the frequency and efficiency of defense means of this aphid with special references to color morphs is rare. We investigated chemical (*i.e.* producing cornicle droplet) and behavioral defense of adult and nymphal stages of green and red morphs of pea aphids when exposed to the predatory ladybird, *Hippodamia variegata* (Col.: Coccinellidae). In addition, differences in pea aphid's defense against adult and larval ladybird were compared. Consequently, red pea aphid showed behavioral defense more frequently than the green, whereas there was no difference between them in terms of chemical defense. Moreover, adult pea aphids exerted more behavioral but less chemical defense. Comparisons on adult and larval defense inducing ability, demonstrated more behavioral defense against adult ladybirds whereas there was no difference between them in provoking chemical defense in the aphids. In spite of a couple of studies suggesting cornicle droplets as an efficient defense against predators, we got into a different conclusion for the given aphid and predator. Therefore, red pea aphid, which is under more predation pressure, applies behavioral defense more frequently. Consistent with previous studies, it sounds that different pressure from predators and parasitoids has caused the two color morphs of pea aphid to evolve different defensive tactics for their different developmental stage.

Keywords: Defensive Behavior, Aphids, Color Polymorphism

PO130

FRUIT-FEEDING AFFECTS FITNESS OF AN OMNIVOROUS ANTHOCORID BUG, *ORIUS STRIGICOLLIS* (HEMIPTERA: ANTHOCORIDAE)

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A growing attention has recently been paid to the importance of non-prey, alternative food sources for biological control agents such as insect predators and parasitoids. *Orius strigicollis*, an omnivorous predator, mainly preys on small insects like thrips and aphids but non-prey foods may be important to sustain or enhance the reproduction particularly when prey is scarce. We tested *O. strigicollis*' responsiveness on sugar-rich fruits to determine the impact of fruit diets on the predator's fitness. Fruits (apple, raisin, banana and strawberries) and commercial Mediterranean flour moth eggs were used to measure difference in *Orius*'s responsiveness to the diets. In non-choice test, *O. strigicollis* responded to all test diets but slightly quicker on strawberries, apple and moth eggs. Residence period on the moth eggs was similar to that of apple and strawberry; females resided longer on these fruits among others but males did not show such differences. In choice test, odor preference was significant when strawberry was paired against apple or raisin but did not differ against or among other fruit pair odors in choice test. The numbers of eggs oviposited were significantly lower for the females fed on fruits than those fed on moth eggs but were greater than control. Fruit provision in the absence of prey increased the predator's longevity. Therefore, fruits can help to sustain the predator's survivorship. Thus, *Orius* predators can use fruits as alternative foods to enhance the fitness when prey is unavailable.

Keywords: Alternative food, food selection, survivorship, fecundity

PO131

INCIDENCE OF GENETIC DIAPAUSE AND "TIME-MEMORY" IN *MICROPLITIS RUFIVENTRIS* WASPS UNDER CONSTANT MASS REARING CONDITIONS

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The solitary koinobiont endoparasitoid *Microplitis rufiventris*, one of the principal wasps of the larvae of the cotton leaf moth *Spodoptera littoralis*, has a diapaus in a portion of each of its several annual generations occurring in the cocooned prepupae or pupae, under constant mass rearing condition. The study present the first ambiguous evidence that diapause in the wasp could be observed under optimum rearing conditions. When continuous mass rearing was done throughout the year at 25/25ºC and 12/12 hours L/D. a small portion of the individuals in large samples of each generation developed diapause while its host does not undergo diapause. The wasps terminated diapause under the same rearing conditions showing different diapause intensities. This has occurred whether the large number of individuals originated from field –collected host larvae or from three successive years of laboratory-rearing parasitoids. The diapause intensity was significantly variable and depended upon the time in the year and was likeness temperature fluctuations outside the lab showing an evidence of "time memory" in wasp behavior. The effect in the lab appears to be independent, or largely so, of environmental conditions. Contrary to non diapaused wasps, diapaused females emerges some days earlier than males. This is the first report of genetic evidence in diapause in *M. rufiventris*. Fecundity of diapaused wasps was diapause intensity-dependent. Wasps diapaused for 64 days developed more mature eggs than either of wasps diapausing for longer period or non-diapaused ones.

Keywords: *Microplitis rufiventris*, optimum mass rearing condition,diapause development, diapause intensity, "time-memory"

PO132

SUPERPARASITISM OR HOST DEFENSE: ADJUSTMENT OF HOST EXPLOITATION STRATEGIES IN RESPONSE TO COMPETITION RISK IN A SOLITARY PARASITOID WASP

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Dinarmus basalis and *Eupelmus vuilleti* are two solitary parasitoid species exploiting larvae and pupae of the bruchid *Callosobruchus maculatus*. When two eggs are laid on one host, only one reaches adulthood, the other being killed during larval competition. When confronted to already parasitized hosts, *D. basalis* females therefore avoid superparasitism and multiparasitism (*i.e.* laying on hosts already parasitized by a conspecific or an allospecific female, respectively). In contrast, *E. vuilleti* females tend to accept already parasitized hosts, even sometimes preferring them to healthy hosts. They also often commit ovicide (*i.e.* killing the eggs already present on hosts) before ovipositing. Under interspecific direct competition, *D. basalis* females outcompete *E. vuilleti* females: they aggressively protect the hosts they exploit for 40 minutes and do more self-superparasitism (*i.e.* lay two eggs during the same oviposition bout), whereas *E. vuilleti* females wait for her opponent to quit the patch and exploit the host. This study aims to investigate whether *D. basalis* females' behaviours under direct interspecific competition are related to their offspring survival chances. *D. basalis* females should defend hosts only during the vulnerability period of their offspring and do more self-superparasitism to increase their chances of winning the larval competition against *E. vuilleti*. To test these predictions, we firstly measured the frequency of self-superparasitism in *D. basalis* under direct interspecific competition. Secondly, we studied the effect of three factors on offspring survival *in situ*ation of multiparasitism: oviposition order (*D. basalis* oviposited first or second), the occurrence of self-superparasitism by *D. basalis* (yes or no) and the time delay between the ovipositions of both females (30-60 min or 3-4 hours). Our results show that, despite the always high survival probability of *E. vuilleti* offspring regardless of the condition, oviposition order and self-superparasitism occurrence influence the probability of *D. basalis* offspring winning larval competition.

Keywords: Interspecific competition, larval competition, *Eupelmus vuilleti*, *Dinarmus basalis*, offspring survival, self-superparasitism

PO133

REPRODUCTIVE STRATEGIES IN APHIDOPHAGOUS LADYBIRD BEETLES AND CONSEQUENCES FOR COMPETITION

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Being widely used for classical and augmentative biological control, aphidophagous ladybird beetles have been broadly studied. In particular, effective pest consumption mostly relies on the offspring foraging activity, and therefore strongly depends on the ladybird reproduction success. Reproductive strategies are very multifaceted and extend from behaviour, *i.e.* host-location ability, to physiology, *i.e.* ovarian dynamism between oogenesis and egg resorption. When ladybird females explore a habitat for oviposition, they perceive odour cues from the plant-prey complex, and may use them as efficiently as they can. For example, some ladybird beetles, being very competitive species, make a better use of the chemical information from a suitable habitat compared to other species. Additionally, plasticity in reproduction allows females to quickly address changes in the environmental conditions (such as collapse of aphid colonies). Exploring plasticity at both species and individual levels is also required. Furthermore, reproductive behaviour is also implicated in the likelihood of competition among juveniles of the predatory species that are part of the same guild. Intriguingly, the peculiar reproductive behaviour of some introduced ladybirds has been linked with the invasive process that these species exhibited in the area where they became established. Therefore, evaluating the ecological consequences of ladybird reproduction would be useful, although challenging. Here we addressed key questions related to the ladybird reproductive strategies, considering the implications for possible direct competition and, ultimately, pest suppression.

Keywords: Coccinellidae, Insect Behaviour

Behaviour

PO134

PATTERNS AND MECHANISMS OF LARVAL AGGREGATION IN *NECRODES LITTORALIS* L. (COLEOPTERA: SILPHIDAE)

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Some larval carrion insects (*e.g.* blowflies or carrion beetles) feed communally on carrion. This aggregation behavior is poorly studied. Here we report results of laboratory experiments on spatial and temporal patterns and mechanisms of larval aggregation in *Necrodes littoralis* (Linnaeus, 1758) (Coleoptera: Silphidae), a carrion beetle frequently breeding in large vertebrate cadavers. Agregation patterns were tested for different instars, various number of larvae (20, 40, 80 or 160) and after different times (5, 15, 30, 60, 180 and 300 minutes). Experiments were made on a layer of meat, in plastic containers put in a cardboard box to prevent light-induced stress. Number of aggregates, number of larvae within an aggregate, number of disaggregated larvae and aggregation index were quantified. Larvae formed aggregates usually at the wall of the container or under the meat. In a no-meat setup aggregates were very unstable indicating that larval food is necessary for the larvae to form stable aggregates. On meat, number of initial aggregates decreased with time, whereas the percentage of aggregated larvae increased with time. Moreover, we found that number of larvae favors aggregation. To explore the mechanisms for aggregation, we focused on chemical cues first. Larvae were put in the container divided into two parts: without a chemical trace (control part) and with chemical cues left by the feeding conspecifics (experimental part). Surprisingly, first instar larvae and to some extent second instar larvae avoided the experimental part of the container, whereas third instar larvae aggregated more frequently and abundantly in this part. Then we focused on the temperature mechanism and tested aggregation along a temperature gradient (20-40°C) using the thermal imaging camera. First and second instar larvae aggregated in a range of 33-38°C, whereas third instar larvae chose two temperature ranges (21-23°C and 30-36°C). Moreover, larval aggregates "followed" the temperature. When temperature in the center of the container was higher, larvae aggregated there, despite their preference towards the container walls. Hence, the simple temperature mechanism may be responsible for aggregation behavior in *N. littoralis*. The third larval stage may additionally follow chemical traces produced by the feeding conspecifics. The study also indicates that larval aggregation may have different background in different larval instars of *N. littoralis*.

Keywords: Carrion, larval aggregation, Necrodes, temperature

PO135

INSECT TRAITS AS PREDICTORS FOR AGE AT EMERGENCE: REFINEMENT OF AGE ESTIMATES IN FORENSIC ENTOMOLOGY

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Forensic entomologists estimate post-mortem interval based usually on the age of a crime scene insects. Insect age is predicted from the developmental stage or size. For this purpose insects are classified or measured, and results are compared against laboratory developmental data, *e.g.* thermal summation data. Resultant physiological age is used to estimate the moment of oviposition through the use of crime scene temperatures and backwards summation of thermal units from insect sampling until oviposition. Although insects reveal large intraspecific variation in development time, the method, as being currently adopted, involves simply the use of the developmental constant for a species, *e.g.* the thermal summation constant. In the paper we advocate the refinement of the approach. We think that traits of insects, *e.g.* their sex or size at emergence may be used to calibrate the developmental data employed for the age estimation. The concept was tested using results of the experiment on *Creophilus maxillosus* L. (Staphylinidae) development, in which growth was monitored at seven constant temperatures, under optimal feeding conditions and with individual rearing of beetles. Because the species revealed significant differences between sexes in the development time, we tested whether the use of sex-specific thermal summation models may improve the accuracy of age estimates. Surprisingly, estimates using sex-specific and general models differed insignificantly. Then, we focused on the size and found that adult beetle length is useful for predicting age at emergence. Moreover, regressing age at emergence and size separately for males and females gave the best models and the largest improvement in the accuracy of estimates using the models. These findings demonstrate that size at emergence and sex of *C. maxillosus* may be used to scale developmental data and eventually to improve the accuracy of age estimates in forensic entomology. At present, we are looking for other traits useful for prediction of insect age at emergence.

Keywords: Forensic entomology, post-mortem interval, insect age, insect size

PO136

COMPETITION BETWEEN *NECRODES LITTORALIS* L. (COLEOPTERA: SILPHIDAE) AND BLOW FLIES OVER CARRION

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In the absence of large mammal or avian scavengers, insects are most efficient carrion reducers. Some of them formed a tight link with carrion. Blow flies (Diptera: Calliphoridae) are probably the most prominent among carrion insects, with a suite of adaptations to immediately locate, colonize and reduce carrion. Under favoring conditions they may reduce large vertebrate carcass into bones in just several days. *Necrodes littoralis* L. (Coleoptera: Silphidae) is a large beetle tightly linked with carrion as well. Similarly to blow flies, it uses carrion mostly for breeding. Moreover, both taxa are dependent on the same resources, *i.e.* carrion soft tissues and for this reason we hypothesize that competition between blowflies and Necrodes shape their adaptations to carrion. By reanalyzing the data from 90 pig carcasses we demonstrate that the communities of *N. littoralis* were distinctly more abundant and long-lasting on cadavers only slightly reduced by blow flies. This finding indicates that *N. littoralis* is adapted to colonize carrion most suitable for its breeding, *i.e.* cadavers being uncolonized or only slightly colonized by blow flies. Moreover, we found that persistence of adult *N. littoralis* on carrion was positively related to persistence of 3rd instar larvae of blow fly *Lucilia caesar* L., a dominant in our studies. This result suggests that adult *N. littoralis* actively reduces population of larval blow flies on carrion. Therefore, we performed several laboratory behavioral assays to get more insight into feeding/killing preference of *N. littoralis*. We found that both male and female adult beetles killed or fed mostly on the late 2nd instar and feeding 3rd instar blow flies. Post-feeding blow flies were killed 4 times less frequent. These findings indicate that *N. littoralis* eliminates competing blow flies through the killing or feeding on these larvae which are most destructive for carrion resources.

Keywords: Carrion, interspecific interactions, competition

Session 3.
Biological Control and Integrated Pest Management

Breeding Invertebrates for Next Generation Biocontrol

PO137

ABOUT THE YELLOW MUTANT OF THE NEOTROPICAL GREEN LACEWING *CHRYSOPERLA EXTERNA*: BIOLOGY AND TRAIT INHERITANCE

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The neotropical green lacewing, *Chrysoperla externa* (Chrysopidae) is a key predator of various pests of small size soft-bodied. Chrysopidae species are known as "green lacewings" due to overall body coloration, with some exceptions in response to the environment. However, yellow mutant individuals were observed emerging from our neotropical green lacewing colony in the laboratory. Thus, the possible implications of this color variation on life history characteristics of *C. externa* yellow mutant were evaluated comparing with its parental green population, as well as the genetic inheritance of yellow trait. In both populations, yellow and green, the biological characteristics were monitored: duration and viability of eggs, larvae and pupae periods, percentage of adults produced and females' fecundity and longevity. Crossing individuals of both populations and progeny evaluation, the hypothesis of an autosomal recessive allele for yellow color was tested, hypothetically considering: yellow=homozygous recessive (yy), green=homozygous dominant (gg) or heterozygous (gy), green offspring=heterozygote (gy) and yellow offspring=homozygous (yy). In addition, backcrossing to ascertain about the inheritance of the yellow color for subsequent generations was performed. The yellow mutant population showed no differences in relation to the green population for any of studied life history characteristics. Mating between heterozygous green progeny (gy×gy) produced 26.74% of yellow offspring, supporting the hypothesis of an autosomal recessive allele (>25%). The yellow color is not a sex-linked inherited, since crosses of yellow females and males with green individuals produced yellow offspring. Regarding the inheritance of the character, an average of 51.23% of yellow offspring was observed in F1 generation. Therefore, we can infer that the allele for yellow color is occurring naturally and at high frequency in the green population, and that the variation in color to yellow does not cause changes in the biology of this predator.

Keywords: Chrysopidae; predator; body color change; backcrossing; homozygous recessive

PO138

INTRASPECIFIC VARIABILITY IN LIFE HISTORY PARAMETERS OF *BRACON BREVICORNIS*

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Intraspecific variability in life history traits is a key to selection for parasitoids that are even more suitable for biocontrol purposes. We analyzed a number of populations of the braconid wasp *Bracon brevicornis*, which is used as a biocontrol agent against several Lepidopteran pest species. This wasp is prone to negative effects of inbreeding, due to its sex determination mechanism: Whenever a female mate with a male that carries a sex determining gene that matches one of her own, 50% of the resulting biparental offspring will become diploid males, instead of females. Since diploid males are essentially sterile in this species, they do not provide any fitness benefit to their mother. They may even more hamper population survival whenever a female mates with them. Since females mate almost exclusively only once in life, accepting a diploid, infertile male as a partner leaves a female constrained to producing only offspring from unfertilized eggs, *i.e.* sons, but no daughters. We found considerable variability in diploid male production under inbreeding conditions between the analyzed populations, which is most likely due to differences in juvenile diploid male survival. This provides a good basis for selection experiments to decrease diploid male survival even more, aiming for a population that will not suffer under the effects of constrained females. We additionally analyzed clutch size decisions of virgin and mated females, offspring survival, response to host size effects, and to previous host encounters, and found pronounced differences especially in the clutch size decisions.

Keywords: Artificial Selection, Complementary Sex Determination (CSD), Biodiversity, Biocontrol, Braconidae, Diploid Male Production

PO139

Poster withdrawn.

PO140

INFLUENCE OF DIFFERENT DIETS ON SOME BIOLOGICAL PARAMETERS OF THE *EPHESTIA KUEHNIELLA* ZELLER (LEPIDOPTERA: PYRALIDAE)

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The Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), is a major pest of stored products, especially damaging cereals. Besides, its eggs and larvae are commonly used for mass rearing of many natural enemies. The production of this moth is not difficult especially at controlled conditions but since it is reared in great numbers, any improvement could reduce costs, space and time. Different diets are suggested in the production of *E. kueniella*, but effectiveness of the diets on the *E. kuehniella* is not clearly understood. In order to assess the effects of diet ingredients on some biological parameters of *E. kuehniella*, we carried out experiments on 10 diets (A-J) as combinations of wheat flour, corn flour, wheat bran, glycerin and yeast. Each diet was prepared as 5 gr and it was placed in the cylindrical transparent plastic containers (5 ×2 cm). Thereafter, 20 eggs were put into the prepared containers and were placed in the climate rooms with 25±1°C, 60±10 RH and 14L: 10D photoperiod conditions. Pupal weight, pupal period, adult ratio and fecundity were determined. The pupal weight ranged from 18 g to 28 g for females, while the heaviest pupae were found in J (wheat flour, wheat bran, glycerin and yeast; 53.33:26.67:15:5%) diet and the lightest pupae were in E (corn flour and glycerin; 85:15%) diet. The pupal period changed from 34 days (Diet I: corn flour, wheat bran, glycerin and yeast; 53.33:26.67:15:5%) to 49 days (Diet E) depending on the diets. Emergences of adults were highest in the H diet (corn flour, glycerin and yeast; 80:15:5%) and were lowest in the E diet. Furthermore, the fecundity of females changed from 122 eggs to 213 eggs and the highest number of eggs obtained from diet J, while the lowest eggs obtained from diet D (wheat and wheat bran; 66.67:33.33%). It is concluded that the diet J was the most efficient for the mass rearing of *E. kuehniella*.

Keywords: *Ephestia kuehniella*, diets; rearing

PO141

THE POTENTIAL FOR SATYRIZATION AS A NOVEL METHOD OF PEST CONTROL

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Mosquitoes are the carriers of many deadly diseases across the world, many without cure or widely available vaccine. Efforts to tackle the disease vector itself are therefore at the forefront of much research. On such proposed pest control method is satyrization, which describes unidirectional hybrid mating between two closely related species that results in severe fitness costs to one of those species (*e.g.* reduced receptivity to further mating, inviable or infertile offspring, infertility). This can be applied via introductions of a closely related species into the population of a more virulent vector of disease, to reduce the population of that vector through hybrid mating. Advantages of satyrization as a pest control method include ecological safety compared with established vector control methods (*e.g.* fogging) and its exemption from GM regulations. The fitness costs associated with satyrization are suspected to be due to incomplete mate recognition from relatively recent evolutionary branching between the two species, and incompatibilities in the seminal fluid proteins when transferred from the male of one species to the female of another. However, little research has been carried out on the underlying mechanisms or its effectiveness for pest control. Using the closely related sister species *Drosophila melanogaster* and *D. simulans*, I have conducted a series of experiments to test the incidence and direction of hybrid mating, the costs involved, and the role of seminal fluid proteins in producing these costs.

Keywords: Hybrid Mating, Pest Control, Mating Incompatibility, Seminal Fluid Proteins

PO142

EFFECTS OF A METAGENOME-SOURCED CHITINASE ON THE PERITROPHIC MATRIX OF LEPIDOPTERA

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The peritrophic matrix (PM) is a thin acellular layer formed by a network of chitin microfibrils, proteins, glycoproteins, and proteoglycans that lines the midgut epithelium of most insects and envelops the midgut lumen content. It plays an important role in the physiology of the midgut and protects the epithelium from toxic materials, pathogen infections, and abrasions due to ingested food. PM is thus considered a good target for pest biocontrol agents able to alter its structure, *i.e.* affecting chitin, proteins or both. In the present study, we tested *in vivo* and *in vitro* the effects of the bacterial 53D1 chitinase on the peritrophic matrix of *Bombyx mori*, a model system among Lepidoptera. This chinitase was isolated from a metagenomic library constructed from an agricultural soil sample. It has been successfully cloned and expressed in the heterologous host *Streptomyces coelicolor* A3(2), from which can be recovered with a high degree of purity. The enzyme is endowed with both exo- and endo-chitinolytic activities and is active in a wide pH range on colloidal chitin - a complex substrate resembling chitin structure in natural sources -, thus suggesting a high versatility. We found that this enzyme has significant *in vitro* effects on the structure of the PM of *B. mori* larvae. A bioassay confirmed these results and demonstrated that the oral administration of the chitinase caused PM alterations, leading to negative consequences on larval development and increasing the percentage of mortality.

This work was supported by the contribution of MAECI (Ministero Affari Esteri e della Cooperazione Internazionale) for the CHITOBIOCONTROL project.

PO143

FITNESS OF *ORIUS MAJUSCULUS* REARED ON ARTIFICIAL FOOD WITH DIFFERENT MACRONUTRIENT COMPOSITION

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Orius majusculus (Reuter) (Hemiptera: Anthocoridae) is a polyphagous predator commonly used for thrips and aphid control. Nowadays its commercial production relies on the use of *Ephestia kuehniella* (Zeller) eggs as a diet. Using this storage pest as a diet eliminates some of the problems associated with tritrophic rearing systems. Although *E. kuehniella* eggs have proven to be a high quality diet for anthocorid bugs and other predators there is still a need for mass rearing the moths which adds complexity to the system. Artificial diets are meant to reduce this complexity, but in the case of *Orius* spp., successful implementation of an artificial diet has not yet been achieved. The aim of the current study was to test the effect of different artificial diets on the developmental and reproductive status of *O. majusculus*. We tested 6 diets with different compositions of lipid and protein and compared the bug's performance with that on a positive control of *E. kuehniella* eggs. Nymphal survival on the artificial diets was acceptable while development was slightly delayed. However, female body weight was lower and fecundity was very low on all the artificial diets as compared with the *E. kuehniella* eggs diet. We also discuss the effect of diet on adult body composition in relation to the differences observed in fitness parameters. Further research focusing on the link between diet content and fitness could yet result in advancing the development of a cost effective artificial diet for this predatory insect.

Keywords: *Orius majusculus*, artificial diet, fitness, fecundity

PO144

EFFECTS OF COLD STORAGE ON ADULT EMERGENCE AND PARASITISM OF *APHIDIUS MATRICARIAE* HALIDAY (HYMENOPTERA: BRACONIDAE)

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Storage at low temperature is an important factor for the mass rearing of biological control agents, especially in augmentative releases of parasitoids and predators. Effective cold storage techniques can reduce the cost of biological control production when the biocontrol agents not immediately needed. Also, cold storage helps to synchronize between field release of biological control agents and critical stages of pest outbreaks. *Aphidius matricariae* Haliday (Hymenoptera: Braconidae) is a solitary parasitoid of more than 40 aphid species and is produced by different commercial companies around the world. The aim of the current study was to investigate the effects of cold storage at 5°C for different periods (5, 10, 15, 20 and 30 days) on the adult emergence and percentage parasitism of *A. matricariae*. The parasitoid was reared on *Myzus persicae* nicotianae Blackman (Hemiptera: Aphididae) established on tobacco plant, *Nicotiana tabacum* L., at controlled environmental conditions (25±2ºC, 70±5% RH and photoperiod of 16:8 h, L:D). One-day-old *M. persicae* nicotianae mummies parasitized by *A. matricariae* were used for storage in darkness condition. Also, control mummies were maintained at 25ºC. In parasitism experiment, 50 individuals of third instar nymphs of *M. persicae* nicotianae were exposed to 1-day-old mated females of *A. matricariae* for 12h. Each treatment was replicated 10 times. According to the results, the percentages of emerged adults in control and 5, 10, 15, 20 and 30 days storage periods treatments were determined to be 91.2, 84.6, 75.0, 66.8, 47.6 and 19.4%, respectively. Besides, the percentage parasitism of *M. persicae* nicotianae by *A. matricariae*, stored for 5, 10, 15, 20 and 30 days were 50.8, 49.6, 42.4, 37.2 and 19.0%, respectively. The results of the current study may be helpful for the mass rearing and mass release of *A. matricariae* to control greenhouse aphids.

Keywords: Temperature, Time period, Cold storage, Parasitoid, Biocontrol

PO145

BIOLOGY, ECOLOGY AND BEHAVIOUR OF *BARYSCAPUS SILVESTRII* (HYMENOPTERA: EULOPHIDAE), A GREGARIOUS PUPAL PARASITOID OF FRUIT-INFESTING FLIES

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Baryscapus silvestrii (Hymenoptera: Eulophidae), a gregarious parasitoid of the olive fly *Bactrocera oleae* (Diptera: Tephritidae), has become the predominant parasitoid in some areas of the Campania region (Southern Italy). However, when it was described, its biology was largely unknown. Initial attempts at establishing a laboratory rearing on the olive fly failed, whereas the medfly *Ceratitis capitata* (Diptera: Tephritidae) turned out to be an ideal alternative host. Having established a laboratory rearing, we could evaluate several biological and ecological traits on different hosts: *B. oleae*, *Rhagoletis cerasi*, *R. completa* (Diptera: Tephritidae) and *Drosophila suzukii* (Diptera: Drosophilidae) were carried out. Choice tests with puparia of different ages and different species were also performed. *Baryscapus silvestrii* completed development only if reared on *B. oleae*, *R. completa* and *C. capitata*. No adult emergence was recorded at temperature of 15°C, while the developmental time from egg to adult decreased with increasing temperature from 20 to 30°C. Furthermore, it was able to develop on puparia of different stages, although it preferred 3-4 day-old puparia. The life cycle of some wasps was much longer than others, but when females were allowed to oviposit for 24 hours only such differences disappeared. *Baryscapus silvestrii* performs destructive host-feeding: the number of hosts killed by host-feeding is similar to the number of hosts parasitized, therefore host feeding doubles the effectiveness of the parasitoid, showing its potential for the biological control of different fruit fly pests.

Keywords: Biological control, host choice, host feeding, Medfly, olive fly, rearing

PO146

Poster withdrawn.

Habitat and Landscape Management to Improve Conservation Biological Control

PO147

NEW TECHNOLOGIES FOR MANAGING PEST POPULATIONS

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New ways of managing pest species are urgently needed as insecticides are lost to resistance and changes to legislation with increasing concern about non-target effects of toxic molecules. However, policymakers have underestimated the difficulty of reducing dependency on pesticides. It requires ecosystem redesign because current crops and cropping systems are highly vulnerable to pests: Crop habitats need to be made less favourable for herbivorous insects but more attractive to natural enemies of the pests. Use of partially resistant crop cultivars can slow down pests while conservation biocontrol can enhance activity of predators and parasitoids. My talk will consider evidence, especially from field experimentation, for or against hypotheses about how insect populations can be managed. It will share insights including *Sitodiplosis mosellana* resistant wheat, use of plant defence activators against aphids, sex pheromones, transgeneic wheat engineered to release the aphid alarm pheromone and *Chilo partellus* stemborer egg induced defence as well as very recent findings with novel nanotech formulations used against *Myzus persicae*. Inducible crop defences have advantages of lower selection for resistance or, with repellents, risk of insect habituation. For example, recent field data show increased parasitism of *C. partellus* in plots of maize with an egg-inducible defence trait. Mechanisms for bringing biocontrol agents out of the margins and into the main crop are needed. Integration of semiochemicals with killing agents can improve targeting of crop protection. There are new opportunities with drones, sensors and robotics. Redesign of agricultural systems will fail if farmers requirements are ignored and including farmers earlier in the evaluation of new technologies may improve chances of success, especially if they can host field trials. Natural ecosystems have survived the onslaught of herbivory for 400 million years and there is much opportunity for learning how insect populations are kept in balance in wild or natural ecosystems.

Keywords: Crops, induced defence, biocontrol, IPM, aphids, stemborers, semiochemicals, nanotechnology, farmers, impact

PO148

ECOLOGICAL FOCUS AREAS TO SUPPORT SUSTAINABLE PRODUCTION AND INTEGRATED PEST MANAGEMENT

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Reform of the Common Agricultural Policy in 2014, saw the introduction of compulsory 'Greening Measures' in an effort to enhance the environmental sustainability of agri-systems. Thirty percent of Basic Payments are now associated with greening measures, chief amongst them being the provision of Ecological Focus Area (EFAs). EFAs apply to tillage farmers with over 15ha of arable land, whereby they must maintain the equivalent of 5% of this arable land as EFAs. Within intensive agricultural landscapes, semi-natural habitats, (including eligible and non-eligible EFAs) support an abundant, diverse community of species, which in turn play an important role in the delivery of essential ecosystem services. These services support production, nutrient cycling, flood regulation, climate regulation, aesthetic value, pollination and biological control of pests. Therefore, conserving and enhancing biodiversity is vital to sustainable agricultural systems. Agroecological approaches are particularly important for arable systems. For example, pest management in cereals is becoming increasingly challenging due to loss of active ingredients in the reregistration process and the development of insecticide resistance. This has resulted in a lack of effective management options and the urgent need to develop more sustainable integrated control options. Increasing the abundance and diversity of natural enemies can enhance their contribution to pest control. Maintaining and enhancing farmland habitats will thus support beneficial species such as natural predators, insect pollinatorsin addition to playing a role in reducing soil erosion and improving water quality. Ongoing work at Teagasc in conjunction with the University of Edinburgh and the Scottish Rural Agricultural College investigates the effectiveness of Ecological Focus Areas to enhance ecosystem services such as pest management and assess their contribution to crop yields.

Keywords: Ecological Focus Areas, ecosystem services, integrated pest management, cereal aphids, natural enemies

PO149

SPATIAL SIGNATURE OF APHIDS, COCCINELLIDS, CARABIDS AND PLANT BUGS IN KARKAJ ALFALFA FIELDS

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Alfalfa is one of the earliest plants domesticated by humans, and has an important role in quality of dairy products. *Therioaphis maculata* (Buckten), *Aphis craccivora* (Koch), *Acyrthosiphon pisum* (Haris) and plant bugs such as *Lygus rugulipennis* Popp and *Adelphocoris lineolatus* (Goeze) are important insect pests in alfalfa fields. In this study spatial signature of these pests and their natural enemies was determined using spatial analysis by distance indices (SADIE), geostatistics, global positioning system and geographic information system. This study was conducted in six alfalfa fields located in the experimental farm of the Faculty of Agriculture, University of Tabriz during 2016 and 2017 growing season. All fields were divided into 20×20 m grids. Weekly sampling was started early in the spring and continued until cutting the hay. Pitfall traps were used for sampling carabids (one trap per grid). Coccinellids were sampled using a 1×1 m quadrat. Two quadrats were thrown at each grid randomly, and the number of pupae, larvae and adult of coccinellids were counted. In order to sample aphids, 20 stems were cut randomly from each grid and shaken into a white pan and were counted. The plant bugs were sampled using six to eight 180° sweeps per grid. The results of geostatistical analysis indicated that the spatial pattern of the studied insects varied during the growing season, and spatial correlation differed from weak (DD <25%) to strong (DD >75%) on different sampling dates. The results of SADIE also largely confirmed the geostatistics results. In order to visualize the spatial distribution of the insects, maps were generated using ArcGIS software. Determining spatial signature of pests and natural enemies provides useful information about their ecology and relationships. This information can be used in biological control and site- specific management of pests.

Keywords: Spatial auto-correlation, Kriging, SADIE, geostatistics, SSIPM

PO150

PROPAGATION IN HETEROGENEOUS LANDSCAPES FOR PULLED AND PUSHED POPULATIONS

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Understanding the expansion patterns of populations is critical for predicting the outcome of global changes or managing the progression of invasive pests. Recent studies have acknowledged the diversity of expansion dynamics, with the description of pulled and pushed populations. Pulled expansions imply that colonization is mostly driven by individuals in small numbers located on the edge of the population, while pushed expansions are characterized by the implication of the whole population in colonization. However, the consequence of these alternative dynamics on expansion patterns in nature remain to investigate. A well-established property of pulled expansions is that their spreading speed depends on population growth rate at low density but is not affected by population dynamics in higher density. In contrast, recent results have demonstrated that pushed expansions could be characterized by a dependency between spreading speed and carrying capacity. We proposed to investigate how these alternative properties affect propagation in heterogeneous landscapes where carrying capacity varies spatially. We chose to address this issue with two complementary approaches: an individual-based model and an experimental laboratory system using *Trichogramma parasitoids* as model species. With both approaches, we compared the propagation of pulled or pushed populations in two different landscapes: a decreasing gradient of carrying capacity, and a periodic alternation of large or small carrying capacity. We found that the spreading speed of pulled populations remained constant in periodic landscapes, and over the majority of the decreasing gradient, while pushed populations displayed a strong sensitivity to habitat structure. Our results provide the first evidence for large-scale, contrasted expansion patterns in pulled and pushed populations and raise exciting perspectives for the management of introduced populations.

Keywords: Heterogeneous landscape, progation speed, pushed expansion, pulled expansion, carrying capacity, microcosms, Trichogramma

PO151

EBIO-NETWORK: A WEB-BASED PLATFORM FOR KNOWLEDGE SHARING ON FUNCTIONAL BIODIVERSITY IN ORGANIC APPLE PRODUCTION

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The web-based platform "EBIO-Network" (European Biodiversity Orchards Network), created within the framework of the CoreOrganic plus project EcoOrchard, provides an interactive communication tool between stakeholders in European organic fruit production at different levels (practice, science, advisory etc.). The website offers the opportunity to share, extract and use stakeholder knowledge in Europe in a participatory approach. As an outcome

of this ongoing exchange, the aim is to provide technical information how to create, keep and assess functional biodiversity in European organic apple production. The platform is hosted as thematic portal at the Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, one of the partners of the EcoOrchard project and is available at https://ebionetwork.julius-kuehn.de. Useful literature is compiled in a database which is regularly updated. Technical sheets and other media (*e.g.* technical videos on-site, reports from field visits) describing methods used maintaining and assessing for functional biodiversity orchards is elaborated by the EcoOrchard team and interested stakeholders. The official language is English; however, we intend to create also country-specific web pages where material in the particular language could be found. The possibility to communicate via a registration tool and a discussion forum as well as during regular meetings will allow adapting the content to the various needs of stakeholders. We hope that due to its open nature the EBIO-Network will be a long-lasting vivid tool for information, exchange and discussion on functional organic fruit production.
Acknowledgements: We thank the team of EcoOrchard for contributions. The German partner is funded by the "Bundesministerium für Ernährung und Landwirtschaft im Rahmen des Bundesprogrammes Ökologischer Landbau und anderer Formen nachhaltiger Landwirtschaft (FKZ: 2814OE005)". The authors acknowledge the financial support for the project EcoOrchard, provided by transnational funding bodies, being partners of the FP7 ERA net project, CORE Organic Plus, and the cofund from the European Commission.

Keywords: Functional biodiversity, ecosystem service, fruit pest, natural enemies

PO152

PARASITOIDS OF *CYDIA POMONELLA* OVERWINTERING LARVAE

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The influence of parasitoids on the reduction of overwintering larvae of *Cydia pomonella* (Lepidoptera: Tortricidae) was monitored in 7 apple orchards in the Czech Republic in 2008-2014. The larvae were collected in each locality for 1-5 years (total 17 samples). The *C. pomonella* larvae were collected in autumn from cardboard belt traps placed on the tree trunks during July. The collected *C. pomonella* larvae were stored in plastic boxes in 5°C in climabox till April of the next year. Parasitism rate was calculated as parasitoids/(parasitoids+*C. pomonella* larvae)*100. Dead caterpillars were not dissected. Tachinidae were determined only to family. In total, 9 Hymenoptera species were reared from *C. pomonella* larvae. Theroscopus hemipteron (56% from the all parasitoids) and *Trichomma enecator* (20%) were the most common species. Ascogaster sp. (6%) and Tachinidae (6%) were important only in several localities. Parasitism rate ranged between 0.3-62.5% and depends on the year and locality. The parasitism rate was underestimated in some localities due to caterpillar collection by growers who do not collected (recorded) larvae parasited by ectoparasitoids (*T. hemipteron* & Chalcidoidea). The parasitism rate was usually low and had no significant effect on the reduction of *C. pomonella* population in the next year. Nevertheless, combination of parasitoids, other natural enemies, selected insecticides and alternative methods for *C. pomonella* control (*e.g.* mating disruption, CpGV, *Bacillus thuringiensis*) could provide sufficient efficacy in IPM or organic orchards. Parasitoids are an important part of antiresistance strategies.
Acknowledgement: This work was partly supported by project QK1710200 and RO 0417.

Keywords: *Cydia pomonella*, parasitoids, Ichneumonidae, Braconidae, Chalcidoidea, Tachinidae

PO153

SUSTAINABLE FRUIT PRODUCTION – BIODIVERSITY ENHANCEMENT

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Sustainable agriculture is a way of farming, which is based on knowledge of ecosystem services, and understanding the relationships between organisms and their environment. Improvement of the current integrated or organic production systems against pests is based usually on using narrow-spectrum insecticides instead of the non-selective ones. This is a good start, nevertheless it is not enough for increase of the ecosystem services and local biodiversity. Diversity of insects depends on the diversity of plants, which is usually poor in agrocenoses. One plant species hosts 10 insect species on average in the Czech Republic. More plant species brings more insect species into the locality. We tested different flowering-plant mixtures in the middle of inter-rows in apple orchards in Bile Podoli in 2013–2017. An influence of flowering inter-rows was compared with standard, frequently mowed inter-rows, with the dominance of grasses. Monitoring of insect species was done by repeated transect counts and visual observations of the plant mixtures. Blooming plants in inter-rows had positive effect on the occurrence of all selected groups of insects (predators, parasitoids, pollinators, Rhopalocera). Direct positive effect on the reduction of pest populations was observed only in aphids, which were killed by Coccinellidae. The impact of other beneficials is difficult to prove, due to their high mobility within the area, where an influence of landscape is more important than few flowering inter-rows. Nevertheless, the higher occurrence of insects in the flowering inter-rows indicates the way how to improve the (functional) biodiversity in orchards in general (not just apples). A classical IPM program was performed during the whole season in the orchard, and no negative effect of the insecticide use was observed on colonies of *Apis mellifera*. There is too much emphasis on this species, which hampers the increase of biodiversity in agrocenoses.
Acknowledgement: This work was supported by project Danone Early Life Nutrition - Project Socrates' and RO0417.

Keywords: orchard, plant diversity, functional biodiversity, Rhopalocera

PO154

ENVIRONMENTAL VARIABLES AT MULTIPLE SPATIAL SCALES SHAPE PARASITOID ASSEMBLAGES IN AGROECOSYSTEMS

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The abundance of parasitoids on plants is affected by local plant-scale factors, as well as by variables that characterize broader spatial scales. We evaluated the effects of local host density (a small-scale variable), habitat (intermediate scale) and location along a climate gradient (large scale) on parasitoid assemblages. Ten species of chalcid parasitoid wasps (families: Aphelinidae, Mymaridae and Trichogrammatidae) and their hemipteran hosts (whiteflies, leafhoppers and Delphacidae planthoppers) were investigated. Arthropods were suction-sampled from 13 sites across a 250-km climate gradient in Israel. Each study site comprised a pomegranate orchard, which was sampled along with its neighboring orchards and natural habitats. Several samples were collected from each habitat for the small-scale analysis. In five of the parasitoid species, population sizes were affected only by the local abundance of a single host species, with no preferences for a particular habitat or climate. Three additional species were affected by both habitat and local host abundance. These parasitoids occurred mainly in the natural habitat, even though their hosts were more abundant in the orchards. The two remaining species were most abundant in the herbaceous vegetation inside the agricultural plots, as were their hosts. None of the studied hosts preferred natural areas to agricultural ones, and none of the sampled parasitoid species preferred the orchard trees to non-crop vegetation. Only one of the ten parasitoids was affected by the climate gradient – it was more abundant in drier areas than in mesic ones. These findings demonstrate the complex interplay of factors at different spatial scales that shape parasitoid assemblages. Habitat-scale variables can be just as important as local plant-scale conditions, such as host densities, for some parasitoids. Additionally, the results suggest a predominance of plant-scale and habitat-scale variables over climatic factors in shaping the studied species assemblage.

PO155

TEMPORAL CHANGES IN SPATIAL DISTRIBUTION OF CARABID BEETLES AROUND ARABLE FIELD-WOODLOT BOUNDARIES

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Carabid beetles are considered to be beneficial arthropods suppressing pest and weed seed abundances in various agroecosystems. Good knowledge of spatial distribution of carabid beetles in agricultural landscapes is crucial for efficient management of ecosystem services provided by this group of insects. In the present study, we investigated spatial distribution of carabid beetles around arable field-woodland boundaries throughout the vegetation season. The study was performed in the model area near Sedlčany, Czech Republic, using pitfall traps arranged along four transects perpendicular to arable field-woodlot boundaries and including woodlot interior as well as open field matrix. Carabid spatial distribution was analysed during four sampling periods: early spring, late spring, summer and late autumn. Spatial distribution of carabid activity-density and species richness was not stable in time. Total carabid activity-density was highest within arable fields with the exception of early spring season when total activity-density peaked at the ecotone. Observed pattern was driven mainly by the spatial distribution of open habitat species, which aggregate near arable field edges during the winter and stay there in early spring. Open habitat species also penetrate into woodlots during the summer season, but occur almost exclusively outside woodlots in other sampling periods. Activity-density of forest species was highest within woodlots with the exception of early spring season, when their activity-density peaked at the ecotone. Carabid species richness was the highest within arable fields in close proximity to arable field-woodlot boundaries with the exception of summer season, when total species richness was evenly distributed. Significant spatiotemporal variability was observed for species composition of carabid assemblages. Occurrence of open habitat species and habitat generalists tend to be more variable in time than occurrence of forest species.

Keywords: Carabidae, Coleoptera, diversity, ecotone, spatial distribution

PO156

HABITAT MANIPULATION TO ENHANCE NATURAL ENEMY ABUNDANCE IN SPANISH ORANGE

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Citrus production is affected by a diverse range of pest species, many of which are successfully controlled using classical biological control. However, for the effective control of some pest species, insecticide application is still required. To support sustainable intensification and a reduced reliance of insecticides, this study investigates whether the provisioning of a noncrop habitat in Spanish orange orchards can enhance the abundance and diversity of natural enemies and control these key pests. This approach, termed Conservation Biological Control (CBC) has been used effectively in other crops including fruit trees, cereals and olericulture crops, but this is the first study in which an engineered habitat, comprised of two grass species and twelve forbs species, has been investigated in citrus. Across four citrus farms in Huelva, four replicate blocks were used to investigate two different management strategies for alleyways between rows of trees; 1) a control treatment in which alleyways were managed conventionally throughout the season, and 2) a CBC habitat in which a seed mix was established in alleyways between rows of orange trees. From May to October 2017, natural enemies were sampled from citrus tree canopies and from alleyways between rows of fruit trees using suction sampling and sweep netting, respectively. Preliminary results indicate that the abundance and diversity of natural enemies both from the canopy and the alleyways are enhanced by the presence of the CBC habitat. These findings suggest that despite differences in community composition between orange tree canopies and alleyways, the natural enemies typically associated with the citrus tree canopy are likely to benefit from the sown resource.

Keywords: Citrus, Conservation Biological Control, ground cover, habitat management, predator, parasitoid

PO157

EFFECT OF NON-CROP VEGETATION ON PARASITOID DIVERSITY IN VINEYARDS OF ISRAEL

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Conservation of natural vegetation within vineyards is an emerging agricultural practice that offers multiple benefits such as reduction of herbicide use, protection from soil erosion, soil enrichment, control of water quality, aeration and regulation of soil temperature. The preservation of the non-crop vegetation might also enhance Conservation Biological Control due to the resources that it provides to local natural enemies. However, the consequences of this approach for pest populations are often not well characterized. Our study tests the effects of non-crop vegetation on arthropod diversity in vineyards in Israel, while focusing on parasitoid wasps as natural enemies of vineyard pests. We suction-sampled arthropods throughout the vine growing season in four vineyard pairs of similar age, grape variety and insecticide application, but different vegetation management treatments: (1) repeatedly spraying herbicide throughout the vineyards, and (2) spraying directly under the vines, letting natural vegetation grow between the vine rows and trimming it only towards the end of the growing season. The main potential pest groups found were thrips, mites, leafhoppers (mainly Empoasca) and moths; the main groups of natural enemies were parasitoids and spiders. The most dominant species/morphospecies of parasitoids were *Telenomus* sp. which commonly attacks true bugs and moths; *Oligosita* sp. which parasitizes plant and leafhoppers; and Lymaenon litoralis which is an important leafhopper parasitoid. Both vegetation composition and the composition of the most

common parasitoid species differed among treatments. In addition, we found that the effect of treatment varied significantly along the season. Namely, in April parasitoids were more abundant in the vegetation cover plots and in July in the plots without vegetation cover. These preliminary results suggest that the effect of vegetation treatment is time-dependent, which is potentially relevant for the management of the non-crop vegetation for biological control purposes.

Keywords: Conservation biological control, parasitoid diversity, non-crop vegetation, natural enemies, vineyards

PO158

OCCURRENCE OF PEST AND NATURAL ENEMY IN THE FIELD OF PEPPER PLANT ACCORDING TO VEGETATION MANAGEMENT

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Pepper is one of the most important vegetable crop in Korea. There are many pests such as aphids, tobacco moths, etc of pepper. In order to investigate the effect of insect pest control on vegetation management in pepper cultivation, the density of pests and natural enemies on pepper field was investigated according to companion plant cultivation. This test was carried out in the organic cultivation field on the National Institute of Agricultural Science, Wanju city, Jeonbuk Province. One experimental section was seeded with three green manures (hairy vetch, alfalfa, crimson clover) and three kinds of flower plants (zinnia, buckwheat and cornflower) before the pepper was planted. The other experimental section was covered plastic film during pepper cultivation. the density of aphids at the section covered with film was higher at the section with companion plants from mid May to the end of June. The density of spiders at section with companion plants was higher, but the density of ladybugs at section covered film was higher than the other section.

Keywords: Pepper, pest, natual enemy, vegetation management

PO159

EFFECTS OF GREEN COVER ON THE BIODIVERSITY OF ARTHROPOD WITHIN A MEDITERRANEAN VINEYARD

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Habitat management can reduce or prevent pest density by enhancing functional biodiversity and associated ecosystem services. An interesting strategy in sustainable agriculture is managing and increasing the arthropod biodiversity by green covers. In this study, we have assessed the effect of ground cover management (tillage, native cover crop and flowering cover crop) on the total and functional abundance of arthropods in a vineyard of La Rioja (Spain) from May to September 2016. The native cover crop was characterized by the presence of *Bromus tectorum*, *Capsella bursa-pastoris*, *Conyza* sp., *Galium aparine*, *Hordeum murinum*, *Melilotus* sp., *Stellaria media*, *Urtica* sp. and *Veronica hederifolia*. The flower cover was composed mostly by *Calendula officinalis*, *Centaurea cyanus*, *Cosmos bipinnatus* and *Eschscholzia californica*. Arthropods were sampled using pitfall traps and vacuum sampling of canopy and green cover. More of 15.000 arthropods were collected. They belong to Araneae, Coleoptera, Diptera, Hemiptera, Hymenoptera, Isopoda, Myriapoda, Neuroptera, Opiliones, Orthoptera and Thysanoptera orders. There are differences between relative abundances of the arthropods orders collected in both sampling methods: mainly Hymenoptera and Coleoptera by pitfall traps, and Hemiptera, Hymenoptera, Diptera and Thysanoptera by vacuum sampling. The presence of native cover crop significantly affected total abundance of arthropods, including natural enemies (Carabidae and Ichneumonoidea), which can provide ecosystem services in vineyard. However, this difference was only observed between treatments in the case of pitfall traps. Furthermore, Coleoptera and Hemiptera were significantly more abundant in native cover crop. At lower taxonomic levels, the native cover crop significantly increased the abundance of Carabidae, Cicadellidae and Ichneumonoidea. On the other hand, green cover showed higher Cicadellidae abundances.

Keywords: biodiversity, green cover, arthropods, pitfall traps, vacuum sampling

PO160

SOLANUM NIGRUM (SOLANACEAE): AN IMPORTANT RESERVOIR OF PARASITOIDS AND HYPERPARASITOIDS IN WEST AND SOUTH REGION OF TURKEY

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The subfamily Aphidiinae includes parasitoid species that are of high important for the biological control of aphids. The present study aims to shed light on the aphidparasitoid-hyperparasitoid interactions that are related to *Solanum nigrum* (Solanaceae), and their seasonal abundance in East Mediterranean (Adana, Gaziantep, Hatay, Mersin, and Osmaniye), West Mediterranean (Antalya, and Karaman), and Eagan Region (Balıkesir, Çanakkale, İzmir, and Muğla) of Turkey. Totally, 3849 aphid colonies were sampled from different cultured plants, weed, and shrubs. Among them, 592 aphid colonies originated from *Solanum nigrum*, 85% of which were found parasitized during 2001-2013. We recorded very high parasitoid and hyperparasitoid density occurring on *Solanum nigrum* during January, November, and December. This issue shows that *S. nigrum* is an important refugee for parasitoids and hyperparasitoids when other plants do not host aphids during the winter time. The non-economic important *Aphis fabae* sollanella was the predominant aphid that attacked *S. nigrum*. Totally, 19,183 parasitoid individuals were identified. The parasitoids *Aphidius colemani*, *Binodoxys angelicae*, *Lysiphlebus confusus*, and *Praon volucre*, while the hyperparasitoids belonged to Cynipoidea and Chalcidoidea were the most abundant taxa that were emerged from *A. fabae* solanella. Our results indicate that *Solanum nigrum* is important reservoir plant species that functions as banker system in the sampled region. Preservation of *S. nigrum* in non-cultivated areas could potentially enhance the biological control of noxious aphids attacking the adjunct cultured plants in the region. *Solanum nigrum* is biannual shrub that grows under big trees and shadows. It is not considered economically significant weed for agriculture, however it is important for successful biological control of aphids in the region.

Keywords: Black nightshade, Aphidiinae, Aphelinidae, Aphididae, Turkey

PO161

CAPSELLA BURSA-PASTORIS L. (BRASSICACEAE): AN IMPORTANT OVERWINTERING HOST PLANT OF THE APHIDS IN MEDITERRANEAN AND AEGEAN COSTAL AREA OF TURKEY

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Aphids reproduction is a complex issue and depends on the where they live; a phase of sexual reproduction generally occurs in temperate regions while no male or no eggs production take places in subtropical and tropical area. This asexual production also depends on overwintering hostplant and the plant plays vital role for successful expansion in spring population. In the present study, the survey were taken a place to determine overwintering host plant of economically important aphid species, and their parasitoids in Mediterranean and Aegean costal region of Turkey, from November to March during 2001-2013. In the survey, 2596 plant material were taken to determine what kind of aphid species were living on them during the project. Our survey reveal that 28 aphid taxa were determined on 23 weed species. *Capsella bursa-pastoris* (L.) (Brassicaceae) (41.06%), *Solanum nigrum* L. (Solanacea) (%35.98), and *Vicia faba* L. (Fabaceae) (%9.51) are recorded as most common sampled weed species while *C. bursa-pastoris* have 21 different aphid species and *S. nigrum* and *V. fabae* have only three and two, respectively. Although *Amaranthus retroflexus* L. (Amaranthaceae) (1.96%), *Sinapis arvensis* L. (Brassicaceae) (0.96%), and *Urtica urens* L. (Urticaceae) (2.58%) has very a low sampling frequency, they are the host plants of six, seven, and six aphid species. This results clearly show that *C. bursa-pastoris* is the key host for overwintering of Aphididae such as *Aphis fabae* Scopoli, *Aphis craccivora* Koch, *Aphis gossypii* Glover, *Brevicoryne brassicae* (L.), *Lipaphis erysimi* (Kaltenbach), *Rhopalosiphum padi* (L.), and *Rhopalosiphum maidis* (Fitch). Moreover this aphid colonies on the *C. bursa-pastoris* is also become source for parasitoids and hyperprasitoids. Totally, 12,014 parasitoid and 444 hyperparasitoids individuals were identified. The parasitoids *Aphidius colemani*, *Aphidius matricariae*, *Binodoxys angelicae*, *Diaeretiella rapae*, *Ephedrus persicae*, and *Lysiphlebus confusus* were the most abundant taxa that were emerged from the aphids on *C. bursa-pastoris*.

Keywords: Shepherd’s purse, Aphididae, Overwintering host plant, Aphidiinae, Aphelinidae, Mediterranean

PO162

HABITAT USE BY CROP PESTS AND NATURAL ENEMIES IN A MEDITERRANEAN VINEYARD AGROECOSYSTEM

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Conservation of natural habitats within agroecosystems often enhances the abundance and species diversity of beneficial arthropods, but may also support agricultural pests. The resulting impacts on crops can be affected by habitat preferences of pests and their potential natural enemies. We studied the spatial distribution of several potential grapevine pests (European grapevine moths, grapevine mealybugs, leafhoppers and aphids) and of their most abundant natural enemies (parasitoid wasps and spiders) in a Mediterranean wine-producing vineyard agroecosystem in northern Israel during 2015. Arthropods were sampled in five vineyards (from vines and from herbaceous vegetation), and from adjacent natural habitats, using pheromone traps, visual searches and vacuuming. Grapevine moth and mealybug populations were highest inside the vineyards, furthest away from natural habitats. Leafhoppers were equally abundant in all parts of the vineyards and in adjacent natural habitats, while aphids mostly inhabited the herbaceous vegetation within vineyards. The proportion of natural enemies out of all arthropods was highest in the natural habitats. Parasitoids were common (14% of all the suction-sampled arthropods) and diverse (168 morpho-species). Parasitoid diversity was highest in natural habitats, intermediate on herbaceous vegetation within vineyards, and lowest on vines, and their abundance was higher on herbaceous vegetation than on the vines. The parasitoids’ distribution between natural and agricultural habitats varied among morpho-species, and their community composition differed significantly among habitats and sampling dates. Spiders were less common than parasitoids (2.8% of all the suction-sampled arthropods), yet similarly distributed, with significantly higher abundance and diversity on herbaceous vegetation than on the vines. Thus, natural enemies in our study system exhibited a stronger preference for herbaceous vegetation than did grape pests. We therefore suggest that conservation of non-crop herbaceous vegetation around and within vineyards is likely to benefit natural enemies of vine pests.

Keywords: Arthropod diversity, non-crop vegetation, parasitoids, spiders

PO163

DIFFERENT PERMANENT PRESERVATION AREAS OF THE PAMPA BIOME AS A RESERVOIR OF PREDATORY MITES

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The Pampa Biome harbors a high diversity of fauna and flora, which maintain the stability of the ecosystem and provide abundant biological resources for economical use. A rich biodiversity of predatory mites can increase the production of neighboring agricultural ecosystems. The present study has the aim to identify which are the predatory mite species associated to different environmental recovery areas of the Pampa Biome, Brazil. Moreover, the study investigates whether predatory mites might be considered bioindicators. For this study different environments (Forest, Grassland, fenced) protected for more than 12 years were selected, with three repetitions for each studied area. The data were compared with ANOVA at a significance level of 5%. The species composition was evaluated with PerMANOVA and IndVal analysis. Among the 55 plant species evaluated, 30 presented predatory mites. *Elephantopus mollis* Kunth and *Ruellia sanguinea* Griseb presented more than five species of predators. A total of 22 species of Phytoseiidae and 5 species of Stigmaeidae were identified. Some of the species of predators collected, such as *Agistemus floridanus* Gonzalez-Rodriguez, *Ambliseius tamatavensis* Blommers, *Phytoseiulus macropilis* (Banks, 1904) and *Typhlodromalus aripo* De Leon, are already used in biological control programs. The Forest environment presented a different species composition as fenced and Grassland environments. The abundance of predatory mites per environment, on average, was higher in Forest (27.67±5.08) and lower in fenced (10±5,25) and in Grassland (2.83±0.83). Of the 27 species, *Amblydromalus limonicus* (Garman & McGregor,) and *A. tamatavensis* were significantly associated with the Forest environment. *Zetzellia malvinae* Matioli, Ueckermann & Oliveira, was associated with the Grassland environment. The preservation areas of the Pampa Biome serve as predator's reservoir. The fenced as a measure of environmental recovery was not satisfactory, since it is not characterized as forest and lost some characteristics of the grassland environment.

Keywords: Biome, Biological Control, Phytoseiidae, Stigmaeidae.

PO164

SEASONAL DYNAMICS OF COMMON GREEN LACEWING (*CHRYSOPERLA CARNEA* STEPHENS, NEUROPTERA, CHRYSOPIDAE) IN THE FIELD WITH SPRING WHEAT

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In 2013, the seasonal dynamics of common green lacewing (*Chrysoperla carnea* Stephens) was investigated. At the Laboratory Field of Biotechnical Faculty in Ljubljana we placed 3 traps with synthetic lure in the field with spring wheat. The traps were intended for allurement of the common green lacewings females, which deposited eggs on the black scent bags. From the last 10-day period of May until the first 10-day period of August we counted the eggs on the traps, while in spring wheat in 3 different distances from the traps (1, 3 and 5 m away) we counted the winged and non-winged aphids as well as the mummies parasitised by aphid parasitoids. Capsules with synthetical tractant, the main part of the traps, were changed in monthly intervals, while the eggs on the black scent bags and the wooden bearers, on which the traps were attached, were counted weekly. We found out that the eggs of the common green lacewing occurred on the traps from the first (May 21) until the last counting (August 7), and the irpeak was established on July 2, when more than 65 eggs were recorded per trap in average. It is interesting that only a few eggs of the predator in question were found in spring wheat, however the highest number of non-winged cereal aphids (about 13 aphids/plant) was recorded on this cereal on June 18, and the highest number of winged cereal aphids (about one aphid/plant, mainly on the ears) was found on July 2. Therefore in spring wheat then on-winged aphids were more numerous until the middle of June, and there up on winged aphids prevailed. The mean number of the mummies parasitised by aphid parasitoids increased with the length from the traps, however the highest number of mummies (one/plant) was recorded at the end of June.

Keywords: Common green lacewing, eggs, spring wheat, seasonal dynamics, synthetic lures

PO165

ANALYSIS OF THE PREDATOR INSECT COMMUNITY IN A VITICULTURAL LANDSCAPE

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Intensive and productivist agriculture expansion represents one of the biggest threats to the biodiversity, which leads to a landscape simplification. This, in turn, has a deep negative impact on the ecosystem's functionality and the environmental services and, in particular, the loss of functional biodiversity concerning the natural enemies of pests. Thus, it is crucial to reduce this loss using effective conservation strategies, such as the management of the agricultural landscape. The aim of this study was to evaluate the alfa and beta diversity of the community of insect predator families in different habitats of a viticultural landscape (forest, shrubland, olive grove, grassland and vineyard). For this, combi (a combination of windows and chromatic traps) and pitfall traps were used. Alfa diversity was evaluated by Simpson and Shannon Indexes, while a similarity dendrogram using Bray-Curtis Index was employed to obtain beta diversity. To complete this analysis, a Principal Component Analysis (PCA) was conducted. Alfa diversity assessment showed no significant differences between habitats for Simpson and Shannon Indexes. Regarding beta diversity, the similarity dendrogram showed three habitat groups according to the community structure of predator families: vineyard and olive grove (92% of similarity); shrubland and forest (75% of similarity); and grassland (36% of similarity with the other two groups). The PCA demonstrated that habitat (component 1) and season (component 2) were the main factors of ordination, which explained 20% and 13% of the variability, respectively. Furthermore, it was possible to notice correlation between some families and these components. Those include: Staphylinidae and Carabidae present in vineyard and olive grove mainly in May; or Coccinellidae, Aeolothripidae and Formicidae in grassland, vineyard and olive grove in June and July; or Vespidae in shrubland and Cecidomyiidae in forest mainly in June.

Keywords: Alfa diversity, beta diversity, predators, viticultural landscape.

PO166

THE IMPORTANCE OF SEMI-NATURAL HABITATS IN THE AGRICULTURAL LANDSCAPE AS OVERWINTERING SITES FOR BENEFICIAL ARTHROPODS

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Different types of semi-natural habitats in the agricultural landscape can greatly contribute to the provision of regulating ecosystem services such as pest control. Field edges with diverse vegetation that include flowering plants and tussock grasses provide habitats and resources for invertebrates, such as mating and oviposition sites, shelter, overwintering sites, food resources and an environment protected from agricultural operations. Natural enemies of agricultural pests can play an important role in biological pest control by regulating the abundance of pest populations. Predatory arthropods as well as hymenopteran parasitoids are the main natural enemies of pests occurring in crop fields. In this study, we aimed to assess the importance of different agricultural landscape elements as overwintering sites for predatory arthropods and hymenopteran parasitoids. We measured and compared the abundance of different guilds of overwintering natural enemies in landscape elements which were bordering arable fields, to find out whether they have any overwintering site preferences. Five different landscape elements (grassy linear, woody linear, cover crop edge, grassland edge, woodland edge) were studied in five one-km radius landscape circles. Each circle contained all five types of landscape elements. Entomological samples were collected with emergence traps. Within each emergence trap a pitfall trap was used to catch ground dwelling arthropods and a photo-eclector at the top was used to catch hymenopteran parasitoids. Predatory arthropods were identified to four major groups: ground beetles (Carabidae), rove beetles (Staphylinidae), spiders (Araneae) and harvestmen (Opiliones). Hymeopteran parasitoids were identified to family level. According to results, landscape element type influenced the abundance of overwintered predatory arthropods as well as parasitic wasps. The greatest number of predatory arthropods emerged from linear grassy elements whereas the most numerous parasitoid guild was found in grassland edges.

Keywords: Predatory arthropods, hymenopteran parasitoids, ecosystem services, natural enemies

PO167

STUDIES ON THE EFFECTS OF LANDSCAPE DIVERSITY ON PARASITOIDS AND APHIDS IN MOSAIC AGRICULTURAL LANDSCAPES IN FUJIAN, CHINA

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Although extensive studies showing that increasing landscape diversity is generally associated with increases in the abundance and richness natural enemies, which, in turn, enhances biological pest control, evidence suggests the picture may be more complex. In this study, our aim was to investigate the effects of landscape diversity on the parasitoids and aphids at different spatial scales. We investigated nine places in Fujian Province, China. Four habitat types (brassica crops, non-brassica crops, forests and urban areas) were assigned to landscape patches in five concentric circles (100, 200, 300, 400 and 500 meters), and landscape metrics (Shannon diversity and the proportions of habitats) around the center of each sampling site were calculated. Five yellow pan traps, 20m from each other, placed in a five-spot dice pattern, were used at each site to sample insects. Our preliminary results show that the different habitats play moderate roles in affecting the parasitoid and aphid abundances at 100m and 200m in agricultural landscapes. The number of parasitoids positively correlated to the proportion of non-brassica and forests habitats within the 100m radius. While the proportion of brassica crops had a significantly negative effect on parastoids. Non-brassica crops affected aphid number positively. Furthermore, both Shannon diversity index and forests impacted parasitoids positively at 200m, whilst parasitoid numbers decreased with the increase of the proportion of non-brassica crops. Shannon diversity positively correlated with aphid numbers at 200m. No impact of urban areas was detectable on aphid or parasitoid numbers. Our results showed that landscape diversity indeed affected the parasitoids and aphids at 100m and 200m radius in our agriculture landscape but landscape elements farther than this distances do not seem to be influential. The increase of forested areas in a complex landscape should be encouraged to improve the pest control.

Keywords: Landscape diversity, spatial scales, parasitoids, aphids, pest control

Exotic and Native Natural Enemies: How to Plan Biological Control and Save Environments

PO168

FEEDING POTENTIAL AND BIOLOGY OF SEVEN SPOTTED LADYBIRD BEETLE *COCCINELLA SEPTEMPUNCTATA* L. (COLEOPTERA: COCCINELLIDAE) ON *SCHIZAPHIS GRAMINUM* UNDER LABORATORY CONDITIONS

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Ladybird beetles both adults and larvae, are well-known primarily as predators of aphids; however, they also prey upon many other soft bodied insects. Studies were conducted to check the biology and predatory potential of ladybeetle on aphid, *Schizaphis graminum* at four constant temperatures 15°C, 20°C, 25°C and 30°C in Integrated Pest Management Laboratory. The functional response to predation toward *Schizaphis graminum* aphids at 25°C was tested. In *Coccinella septempunctata*, the functional response of adult males differed from those of third instars and adult females, but there was no difference between third instars and adult females. Third instars and adult females consumed significantly more *Schizaphis graminum* than did adult males aphids per arena. Third instars adult females significantly more aphids than did adult males on wheat plants with the recommended action threshold of wheat aphids per plant. Egg larval, pupal and adult mortality was tested, maximum mortality was determined at 25°C while minimum mortality was observed at 15°C. Similarly, oviposition period, lifetime fecundity and egg viability were all maximum at 25°C while it was minimum at 15°C. This indicates that adult male and female oviposition period, lifetime fecundity and egg viability were significantly different from each other at four constant temperatures. *Coccinella septempunctata* show high predation capacities and play important role in suppressing wheat aphid populations.

Keywords: Biology, Potential, *Coccinella septempunctata*, *Schizaphis graminum*

PO169

MANAGEMENT OF INSECT PESTS BY ENTOMOPATHOGENIC FUNGI ISOLATED FROM WALNUT SOILS BY GALLERIA BAIT METHOD IN A BIODIVERSITY HOT SPOT

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Food security is one of the prime concerns of present times because of rising consumer demands, receding agricultural land, climatic change and exponential human interference. Among various food plants, Walnuts are included in FAO list of priority plants because of their nutritive value (Gandev, 2007). Although walnuts are of economic importance, outbreaks of various insect pests hamper their production (Khan *et al.*, 2013). Keeping this in concern, wide-ranging experiments were carried out on Walnut pests by the application of entomopathogenic fungi. First part of the work was focused on collection and identification of walnut pests, for which extensive surveys were carried out during 2015-16 in different walnut orchards regions of Kashmir valley. Of the different pests collected, ten pests were identified by studying their morphology, biology, damaging potential and host range. Some of the insect pests like *Chromaphis juglandicola*, *Panaphis juglandis*, *Chaetoprocta odata* and *Cydia pomonella* etc. were reported to inflict major damage to leaves, inflorescence and fruits. In the second step, soil samples were collected from different regions of walnut growing areas of Kashmir. These soil samples were taken to Darmstadt, Germany wherein entomopathogenic fungi were isolated from them by Galleria bait method. A total of seven fungal strains were isolated of which two were identified as *Beauveria* basiana and Isaria fumurosa while the rest of the strains could not be morphologically identified and therefore were subjected to molecular analysis and DNA sequencing. Production and formulation strategies were carried out for two fungal species, *B. bassiana* and *I. fumurosa*. Different concentrations were prepared and tested on fifth instar larvae of codling moth, *Cydia pomonella*, one of the major insect pests on apple orchards in Germany. The results were encouraging, showing effectiveness and potential of these entomopathogenic fungi for their use as biocontrol agents against different walnut pests.

Keywords: Entomopathogens, Insect pests, Walnuts, Kashmir

PO170

REPELLENT ACTIVITY OF PLANT ESSENTIAL OILS AGAINST RICE WEEVIL, *SITOPHILUS ORYZEA* (COLEOPTERA: CURCULIONIDAE)

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Sitophilus oryzea is the most destructive pest of stored grain products. Application of chemicals use to control the infestation but it has several harmful effects on the quality of grains, human health and environment. The objective of study to identify the toxicity of methanolic plant essential oils and optimum concentration on *Sitophilus oryzea*. Four essential oils *Citrus limon*, *Ficus benjamina*, and *Ziziphus mauritiana* were tested against *Sitophilus oryzea* at concentrations 25%, 50%, 75% and 100% with time interval 1, 2 and 3 hours. The experiment was laid out in completely randomized design (CRD) with the three replication for each concentration. The results showed that higher concentration of essential oils resulted in maximum repellency for maximum exposure period as compared to minimum oil concentrations at minimum exposure period. Furthermore, it is concluded that *Citrus limon* and *Ziziphus mauritiana* proved more toxic whereas *Ficus benjamina* was showed least toxic against *Trogoderma granarium* at all the concentrations.

Keywords: Oils, Plant, Repellent, Rice, Weevil

PO171

IMPROVING KNOWLEDGE ABOUT BIOLOGICAL CONTROL OF SCALE INSECTS IN ORNAMENTALS THROUGH COLLABORATION AND RESEARCH SHARING: THE EXAMPLE OF THE FRENCH CASDAR COCHORTI PROJECT: A DECISION SUPPORT TOOL FOR THE RECOMMENDATION

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The French CASDAR project ‘COCHORTI’ focuses to control scale insects in ornamentals and minimize the use of chemicals. This project is declined in 5 complementary aims. 1) Make the inventory of scale insects on ornamentals, with their distribution and the host-plants. Morphobiometric tools and barcoding will be used. This task could make it possible to put in perspective the molecular and morphological results to identify possible taxonomic locks, to highlight cryptic species. 2) Make the inventory of biological control agents. As for scale insects, morphobiometric tools and barcoding will be used. This task is to identify new candidates as biological control agents. 3) From the studies of the biology of scale insects cycles and natural parasitism, treatments will be advised according to the most sensitive stages and the parasitoids presence. The target is to reduce treatments and to increase the ecosystem services of biological control agents. 4) Moreover, the study will help to identify the best cultural conditions to promote (or inhibit) biological agents. Cultural practices will be synthesized, plant by plant, region by region to determine the conditions that favor the development of mealybugs and conversely. 5) Finally, a database and a guideline of recommendations will be realized for the actors of ornamentals produce. The project started early 2017 for 36 months.

Keywords: Scale insects, biological agents, ornamentals, France

PO172

INFECTING THE JAPANESE BEETLE EMERGING ADULTS IN SOILS TREATED WITH *METARHIZIUM ANISOPLIAE* MET52®: A PRELIMINARY SEMI-FIELD STUDY

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The commercial product MET52® is known to be able to kill *Popillia japonica* (Coleoptera, Scarabaeidae) larvae, however it is not known if this fungal strain, spread onto the ground, is also able to intercept and kill emerging adults. This preliminary study aimed to fill the gap by testing Met 52® (constituted of grain kernels of mycosed barley) in semi-field applications. In particular, ten pupae of the Japanese beetle were singly placed at the bottom of ten

plastic containers (4.5 cmx7 cm) filled with about 200 CC of native sterilized soil along with seeds of *Lolium perenne* to recreate similar field conditions and one gram of Met 52® was distributed in each experimental unit (n=30). The same experimental design (without the fungus) was used also for the control (n=30). The duration of the experiment was 12 days since pupae duration is about two weeks. Every experimental unit was daily checked to verify if pupae died. Concerning the Met 52® treatment, dead adults were washed with 1% sodium hypochlorite water solution, rinsed with sterile distilled water, and placed on moistened filter paper in Petri dishes at 25°C to determine the presence of external sporulation. At the end of the experiment, Met 52® reported an overall adult mortality of about 40% while in the control the mortality was about 7%. Considering that the fungus remains longer in the field, although the present work reported only a moderate control, considerations on its potential use in field application are drawn.

Keywords: *Popillia japonica*, entomopathogenic fungus, biological control, insect pest

PO173

HOST RANGE AND PREDATION BEHAVIOUR OF *TETANOCERA ELATA* (DIPTERA: SCIOMYZIDAE) LARVAE, A NATURAL ENEMY OF PESTIFEROUS SLUGS

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Marsh flies (Diptera: Sciomyzidae) are a diverse family which provide valuable ecosystem services, including many species which provide biological control of molluscs which are agricultural pests and vectors of animal and human parasitic diseases. Recent research undertaken at the Applied Ecology Unit (NUI Galway) on the species *Tetanocera elata* has indicated that it may be a suitable natural enemy and biological control agent of the grey field slug (*Deroceras reticulatum*), a widely-distributed pest of gardens and agriculture. However, before this species may be considered as a biocontrol agent, a thorough investigation into its prey range and preference is required. Early instars are obligate mesoparasitoids on *D. reticulatum*, and sometimes on *D. laeve*, but third instar *T. elata* larvae are free-living and predatory, with the potential to become more generalist predators. To date, this kind of analysis has never before been investigated for this species. The present study investigated the realised prey range, preference, and predating behaviours of third-instar *T. elata* larvae using video recording and analysis software. Three common European pest slug species (*D. reticulatum*, *Arion hortensis*, and *Tandonia budapestensis*) were utilised as potential prey for naïve third-instar larvae in choice (n=50) and no-choice (n=38) trials. Trials were run in dark conditions in specialised chambers and recorded using infrared light to avoid a negative phototactic response from both larvae and slugs. Larvae attacked all three slug species across all trial types at similar frequencies, however *D. reticulatum* was attacked most often of the three in choice trials. *Deroceras reticulatum* was the only species successfully killed by larvae in trials (n=6), suggesting it may be a better or more preferable prey species for *T. elata* larvae. Investigation of prey range and preference has implications for the use of *T. elata* in conservation biocontrol within integrated slug pest management schemes throughout Europe.

Keywords: Behaviour, biological control, Diptera, ecology, predation, prey choice

PO174

SEX ATTRACTANTS IN THE INVASIVE POPULATION OF *VESPA VELUTINA NIGRITHORAX*

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Due to its huge invasion potential, habitat flexibility and specialization in honeybee predation, the alien invasive hornet *Vespa velutina nigrithorax* represents a high-concern species under both an ecological and economical perspective. In the light of the development of specific odorant attractants to be used in mass trapping, we used an integrated approach including behavioural assays, chemical and electroantennographic analyses to investigate the possibility that in the invasive population of *V. velutina nigrithorax* reproductive females emit pheromones to attract males, as demonstrated in a Chinese non-invasive sub-species. We focused on the sex pheromone identified in the sternal glands of the non-invasive sub-species and on venom volatiles. Because of the volatility and complexity of their composition, both secretions could potentially allow both a long-range attraction and a species-specific response, decreasing therefore non-target species bycatches. Behavioural assays in the laboratory showed that males respond to sternal glands secretion, but not to the two synthetic constituents reported as sex pheromone in the Chinese subspecies. Results of chemical analyses, electroantennography and behavioural assays have all shown that venom volatiles are instead unlikely candidates as sex pheromones: a) venom volatiles do not differ neither in quality nor in quantity between reproductive females and workers; b) male antennal response is not different between venom volatiles of workers and gynes and, finally, c) males are not attracted by venom volatiles compared to controls. Our results, showing that males are attracted by female sternal glands secretion, similarly to the non-invasive subspecies, but not by venom volatiles, provide a valuable first step to understand the reproductive biology of *V. velutina nigrithorax* in its invasive range and to develop effective and sustainable management strategies for the species. Further research should aim at investigating the actual attractiveness of the secretion and of its constituents under field conditions.

Keywords: Invasive species, chemical communication, sex pheromone, Asian hornet

PO175

VARYING LEVELS OF ACCEPTANCE AND EGG ABORTION OF THE INVASIVE *HALYOMORPHA HALYS* BY DIFFERENT STRAINS OF NATIVE PARASITOIDS

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The brown marmorated stink bug *Halyomorpha halys* (Hemiptera: Pentatomidae) is an invasive pest of global concern affecting many agricultural crops. In the invaded areas (North America, Europe), the impact of native biological control agents is generally low and mostly attributable to generalist species. Native egg parasitoids, for example, often attack *H. halys* eggs but their offspring are unable to develop. The lack of co-evolved relationships between *H. halys* and native egg parasitoids could be reduced over time if native egg parasitoids adapt to the new host, either by avoiding them (reducing behavioural acceptance) or by gaining the capacity to successfully exploit them (increasing developmental success). The likelihood of adaptation and consequences for biological control is expected to depend on the amount of within-and among population and species variation in acceptance, host killing ability (egg abortion), and developmental success. The aim of this study was to investigate the level of acceptance, abortion rate and development success of five strains of each of the native North American egg parasitoids *Trissolcus euschisti* and *Telenomus podisi*. One-week old mated parasitoid females were exposed to an *H. halys* eggs clutch at 25°C. Oviposition behaviour was recorded for 5 hours for each female. Parasitoid emergence was recorded and after the emergence of the nymphs, the unhatched eggs were dissected and classified as aborted, empty, containing nymphs or parasitoids. As expected, all parasitoid strains showed a high (but varying) level of acceptance of *H. halys* eggs, but offspring development was extremely rare. Varying rates of *H. halys* egg abortion due to unsuccessful parasitism were also observed, with more abortion in the egg masses exposed to *T. podisi* compared to those exposed to *T. euschisti*. This study will be useful to understand at which level the genetic variability may influence the different adaptation level of egg parasitoids to the new host allowing the development and implementation of further efficacy biocontrol strategies.

Keywords: Parasitization, Brown marmorated stink bug, invasive species, abortion rate, biological control, population variability

PO176

PREDATOR-PREY MODELS TO DETERMINE IMPACT OF CLIMATE VARIABILITY ON APPLE ORCHARD BIOCONTROL

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A key threat of climate change to ecosystems is the disruption of species interactions. This has implications for integrated pest management which relies on biological control to reduce pest populations while reducing reliance on chemical pesticides. Changes to life-history or behavioural traits at a single trophic level have the potential to disrupt species interactions and alter the efficacy of pest control. Research is needed to pre-empt such changes in response to future climatic conditions. Mathematical models are frequently used to predict the individual responses of pests and natural enemies to climate change, typically using temperature response curves to compare changes in development rates with temperature. However, such models have low capability to investigate how species interactions are impacted by a temperature change. Here we present initial results from a Lotka-Voltera predator-prey model, which we apply to assess effects of temperature on population dynamics in two species of apple pest (Woolly Apple Aphid (*Eriosoma lanigerum*) and Green Apple Aphid (*Aphis pomi*)) and their respective parasitoids (*Aphelinus mali* and *Aphedius ervi*). Our model can be used to identify weather scenarios at which aphid populations are unable to be controlled by their associated biological control. In the future we expect this information can be used to advise on optimal timing of pesticide sprays. The model can therefore be used as a tool to increase efficiency of pesticide use complying with European legislation. Our further research will investigate the effects of temperature extremes such as heat waves on these species interactions and we will collect field data to further validate the model's efficiency.

Keywords: Tri-trophic interactions, Pest Control, Climate Change

PO177

EFFECT OF TEMPERATURE ON THE PATHOGENECITY OF MEDITERRANEAN NATIVE ENTOMOPATHOGENIC NEMATODES (STEINERNEMATIDAE AND HETERORHABDITIDAE) FROM NATURAL ECOSYSTEMS

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Seven entomopathogenic nematodes (EPNs) belonging to three species (*Steinernema feltiae*, *S. ichnusae* and *Heterorabditis bacteriophora*) naturally isolated from Mediterranean countries (Southern Italy and Lebanon) were evaluated for their potential to infest greater wax moth (*Galleria mellonella*) larvae at different temperature under laboratory conditions. The laboratory bioassay was conducted at six different temperatures ranging from 10°C to 35°C. Infective Juvenile (IJs) were put in contact with *G. mellonella* larvae in Petri dishes and mortality rates were recorded after 72 hours. The purpose of the study was to evaluate the temperature range in which the EPNs caused larval mortality; higher mortalities were recorded at 15°C and 20°C. All species failed at lower temperatures except for *Steinernema ichnusae* ItS-SAR4, which caused 7% mortality. At 35°C *S. ichnusae* maintained its infectious activity (24%) along with *Heterorhabditis bacteriophora* ItH-LU1 (38%); both were isolated from Italy and were more efficient at high temperatures than the remaining Lebanese isolates

Keywords: *Steinernema feltiae*, *Steinernema ichnusae*, *Heterorhabditis bacteriophora*, Mediterranean Habitats, temperature, bioassay

PO178

INTERACTIONS AMONG PREDATORS OF THE TROPHIC GUILD OF *APHIS GOSSYPII* (HEMIPTERA: APHIDIDAE): "WHEN TWO IS COMPANY AND THREE IS MULTITUDE".

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The population dynamics of phytophagous insects is greatly determined by the interaction among predators within their trophic guilds. In this work, the interactions among three predator species of *Aphis gossypii* Glover (Hemiptera: Aphididae) were assayed in melon. The objective was to identify the combination of natural enemies with fewer negative interactions among predators and with higher rates of aphid predation. Eight combinations of three predators - *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae), *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) and *Deraeocoris*

punctulatus Fallen (Hemiptera: *Miridae*) - at two levels (presence/absence) were assayed in a complete factorial design with four repetitions. Melon plants in pots and enclosed individually in 1.5 m high muslin cages were placed inside a greenhouse. Each melon plant was infested with 20 aphids. Five females and five males of each predator species were released a week later. Plants were observed weekly for 45 days to count the number of aphids and predators (nymphs and adults) on the upper 10 leaves of each plant. At the end of the trial, the abundance of *A. gossypii* in the plants without predators was much higher than in those where they were introduced. The abundance of aphids was significantly lower when two or three species of natural enemies were combined than when only one was used. However, when *O. laevigatus* and *D. punctulatus* were together the number of aphids was lower than when the three predators were combined (*O. laevigatus*+*D. punctulatus*+*A. aphidimyza*). Several interactions were registered among predator species. For instance, *O. laevigatus* and *D. punctulatus* had a negative effect on *A. aphidimyza*. Besides, a mutual negative interference was recorded between *O. laevigatus* and *D. punctulatus*.

Keywords: Melon, Insterspecific Interactions, Natural Enemies, Biological Control, Intraguild Predation

PO178a

BIOLOGICAL CONTROL OF ECHINOTHRIPS AMERICANUS AND GREENHOUSE WHITEFLIES BY THEIR SHARED PREDATOR *MACROLOPHUS PYGMAEUS* IN GERBERA

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Echinothrips americanus Morgan (Thysanoptera: Tripidae), commonly known as poinsettia thrips, is a relatively new pest that has spread rapidly worldwide and causes serious damage in both vegetable and ornamental plants. In this study we evaluated whether the omnivorous predator *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) can be used to control this pest. Moreover, since the poinsettia thrips commonly co-exists with the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) on gerbera crop, we studied whether *M. pygmaeus* has a preference for *E. americanus* or greenhouse whitefly by assessing predation rates on both pest species when offered mixed in the laboratory and through olfactometer tests. To detect the possible additional role of plant-mediated pest interactions, we also included treatments with single and combined pests on plants without predators. Densities of both pests were reduced to very low levels by their shared predator *M. pygmaeus* either when the pests were present together or alone. Predator-mediated effects between whiteflies and thrips on gerbera plants played only a minor role resulting in slightly higher densities of thrips in the presence of whiteflies and predators in the short-term than in the treatment with only thrips and predators. The plant-feeding behaviour of the shared predator probably stabilized pest control and thereby weakening the predator-mediated effects. However, olfactometer tests showed a clear response of the predators to plants infested by whiteflies but not by thrips. Thus, when the pests are more clustered in a crop, this prey preference might cause stronger effects of a temporary release of predation of thrips because of the preference for plants infested by whiteflies.

Key words: *Trialeurodes vaporariorum*, apparent competition/mutualism, prey preference

PO179

GARDEN GASTROPODS: INTEGRATED PEST MANAGEMENT OF SLUGS AND SNAILS IN UK GARDENS

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Slugs and snails cause significant damage to ornamental and crop plants in UK gardens and are one of the most common pest enquiries to the RHS Gardening Advice service. Control methods are used by gardeners but their efficacy is unclear (cultural methods), there may be effects on non-target species and the environment (chemical controls), or there are perceived barriers to their successful use (biological methods). In order to address gaps in our knowledge, and help gardeners to use integrated pest management strategies against gastropods in their gardens, the RHS has developed a programme of research. One field experiment in collaboration with BASF assessed the effectiveness of metaldehyde, ferric phosphate or a gastropod-parasitic nematode when combined with a cultural control (mulch). The synthetic pesticide metaldehyde performed well most consistently, but the more environmentally friendly options, such as the nematode biological control or the organic slug pellet ferric phosphate gave intermediate or good control, particularly when combined with mulch. This study will inform RHS advice to home gardeners, and enable the development of integrated pest management strategies for gardens that minimise the use of pesticides. Citizen science approaches offer much potential in this area since both gastropods and gardening are accessible to the general public and generate significant enthusiasm. A preliminary project with a small number of schools was done, testing the effectiveness of five barriers to gastropods in a simple experimental design using potted lettuce. This preliminary study will be used to refine the methodology and improve participant engagement before repeating the project with a larger number of participants. This will generate sufficient data to ascertain the effectiveness of some popular gastropod barriers.

Keywords: Gastropods, IPM, horticulture, GPN, cultural control, home gardening, citizen science

PO180

NON-TARGET HOST RISK ASSESSMENT OF THE EGG PARASITOID *TRICHOGRAMMATOIDEA CRYPTOPHLEBIAE* FOR CLASSICAL BIOLOGICAL CONTROL OF THE FALSE CODLING MOTH IN ISRAEL

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The false codling moth *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae), is an invasive species in Israel. In order to carry out a classical biological control program, the African egg parasitoid *Trichogrammatoidea cryptophlebiae* was recently introduced to Israel, and non-target host risk assessment was performed as required. In no-choice bioassays, we determined that *T. cryptophlebiae* was unable to develop in eggs of 4 non-target Lepidopteran species of different families: *Pectiniophora gossypiella* (Lepidoptera: Gelechiidae), *Spodoptora littoralis* (Lepidoptera: Noctuidae), *Ephestia kuehniella* (Lepidoptera: Pyralidae), and *Belenois aurota* (Lepidoptera: Pieridae). In contrast, it developed in 2 Lepidopteran species eggs of the Tortricidae family: *Lobesia botrana* and *Epiblema strenuana*. However, in choice bioassays, *T. cryptophlebiae* significantly preferred to parasitize *T. leucotreta* eggs over the eggs of *L. botrana* or *E. strenuana*. Our data adds two more species to the egg parasitoid *T. cryptophlebiae* host list, and support the assumption that it develops only in moth species of the Tortricidae family. Therefore, the risk that it may attack some beneficial native species, other than Tortricid moths, appears to be quite low.

Keywords: Classical biological control. Invasive species. *Trichogrammatoidea cryptophlebiae*

PO181

PREVALENCE OF APHIDOPHAGOUS HOVERFLIES FROM SINDH PAKISTAN

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Prevalence and species composition of aphidophagous hoverflies was assessed in various areas of Sindh such as Badin, Karachi, Larkana, Nawabshah & Dadu during January – April 2017. Hoverflies are a very important group of insects because their ecosystem services are manifold. Their larvae exhibit a variety of feeding modes, whereas their adults are floral visitors of hundreds of different plant species. Aphidophagous hoverflies are one of the vital groups of hoverflies naturally predators environment play vital role in pest management, because their larvae feeds over insect pest such as (aphids, Jassids, Thirps) while adult are good pollinators. These floral resources enhance the longevity and fecundity of adult dipterous flies. Many syrphid species also have been documented as efficient crops pollinations. Hoverflies are a characteristics feature of the spring season in the subtropical areas of Pakistan where the average temperature and relative humidity range from 23OC to 59% respectively. Adult hoverflies were collected by using insect hand net from different crops such as Wheat, brassica, onion, chili, cheeko and from date palm tree blossom. As a result 423 individuals of 4 species belonging to 3 genera of one subfamily identified on the morphological characteristics. These species are (*Episurphous Balteatus*, *Bacha elongate*, *Spherophora scriptera*) presently study detailed prevalence of aphidophagous hoverflies (syrphidae, syrphinae) has been conducted from January to March 2017 from different localities of Sindh like Badin, Karachi, Larkana, and Nawabshah & Dadu. In these areas different crops, vegetable, and fruit are cultivated such as Wheat, brassica, onion,cheeko,chilli,date palm tree and other vegetable are grow throughout the year and the condition are also favorable for the hoverflies.

Keywords: Prevalence, hoverflies, sindh, Pakistan

PO182

PUSH-PULL STRATEGY FOR CONTROL OF *BEMISIA TABACI* IN TOMATO GREEN HOUSE

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The sweet potato whitefly, *Bemisia tabaci* (Gennadius) are major insect pests that causes crop damage worldwide by piercing leaves, sucking sap and transmitting numerous plant viruses. We surveyed the responses of *B. tabaci* to repellent (push) and attractant (pull) materials for the establish of push-pull strategy. From among the essential oils, Methyl isonicotinate showed the high attractant effect, but ocimene and carvacrol were repellent to on adult *B. tabaci*. The light attraction rates of this whitefly were determined using LED light bulbs exhibiting different wavelengths in the visible and UV spectra. *B. tabaci* was mostly attracted to green LEDs (520 nm), but very was repellent to pink LEDs (450+660 nm mixture). As a banker plant, *Fagopyrum esculentum* Moench showed high promise in hosting natural enemy of whiteflies, *Cyrtopeltis tenuis* (Reuter, 1895).

Keywords: Control, *Bemisia tabaci*, Push-pull strategy, Tomato, LED

PO183

NATIVE AND INTRODUCED PARASITOIDS IN THE BIOLOGICAL CONTROL OF THE CHESTNUT GALLWASP IN SARDINIA

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The Asian chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae) (ACGW), is one of the most damaging insect pest on Castanea species (Fagales, Fagaceae) worldwide. The classical biological control based on the release of the parasitoid *Torymus sinensis* Kamijo (Hymenoptera, Torymidae) is considered to be the only effective method of controlling ACGW whereas native oak gall wasp parasitoids seem to be not able to contain this pest. In order to monitor the native parasitoid community both before and during the diffusion of *T. sinensis*, a multi-year survey was conducted in the Barbagia of Belvì area (Sardinia, Italy). In the period 2012-2014 and in 2016 spring galls were collected from seven sites where *T. sinensis* was not released but naturally spread from neighboured release sites. In total, 4264 galls were dissected and approximately 5,000 parasitoids were identified by molecular techniques or morphological characters. Annual parasitism rates calculated as the mean number of cells occupied by parasitoids per gall were 12.3%, 14.7%, 47.1% and 73.7% in 2012, 2013, 2014 and 2016, respectively, and the contribution of native species to these parasitism rates decreased from 67.8% in 2012 to 2.5% in 2016. In contrast, *T. sinensis* contribution increased since the first year of monitoring, reaching a peak of 96.6% in 2016. Our results show that ACGW galls hosted a rich native parasitoid community until *T. sinensis* was absent or not widespread into the studied area. Hence, the incidence of native parasitoids gradually declined as a result of the specificity and synchrony of *T. sinensis* with ACGW.

Keywords: *Torymus sinensis*, *Dryocosmus kuriphilus*, parasitism rate

PO184

BIOLOGICAL CONTROL OF *POPILLIA JAPONICA* (COLEOPTERA, SCARABAEIDAE) IN ITALY: A SURVEY OF INDIGENOUS ENTOMOPATHOGENIC NEMATODES AND FUNGI

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Popillia japonica (Coleoptera, Scarabaeidae) is an invasive alien insect that was recently spotted in northern Italy. This Scarab beetle is a severe agricultural pest, noxious to more than 300 plant species. Italian Plant Protection Organizations (PPO) of the Regions Piedmont and Lombardy have employed so far biological control strategies in order to manage the outbreak in the Ticino Valley. Within this scenario, the purpose of our study was to investigate potential indigenous biological control agents, with special emphasis on wild species/strains of entomopathogenic nematodes (EPN) and fungi (EPF) present in the infested area. To this end, we analysed 155 soil samples (500 g), collected in perennial meadows and forest areas to isolate local EPNs and EPF. For what concerns the EPNs, we found 36 species belonging to Heterorhabditis (3 strains) and Steinernema (33 strains) genera. Preliminary pathogenicity assays were performed with these strains using *Galleria mellonella* larvae. Afterwards, we tested the seven most performing Steinernema and all the Heterorhabditis strains against third instars larvae of *P. japonica*. As a result, we observed that the Heterorhabditis species caused a mortality in Scarab beetle larvae ranging from 40% to 84%, whilst the Steinernema strains caused at most 52% mortality. As for the EPF, we found 117 strains: 103 belonged to *Metarhizium anisopliae*, 5 belonged to *Beauveria bassiana*, 7 to *Paecilomyces genus* and 2 to *Isaria genus*. *Metarhizium* strains were divided into 11 morphological groups based on the appearance of colonies grown on SDAY. Bioassays were then carried out testing one representative of each group directly against *P. japonica* third instars. In three of the tested strains we observed a mortality of about 90%. However, though promising, these preliminary laboratory results require further confirmation in field experiments.

Keywords: Japanese beetle, biological control, entomopathogenic nematode, entomopathogenic fungi

PO185

HEXAMERMIS POPILLIAE N.SP.: A NEW MERMITHID NEMATODE PARASITIZING THE JAPANESE BEETLE *POPILLIA JAPONICA* NEWMAN IN ITALY

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Popillia japonica (Coleoptera, Scarabaeidae) is an invasive pest particularly harmful to agricultural environments and difficult to control. Since 2014 this quarantine pest is also present in northern Italy along the Ticino Valley between the Regions Piedmont and Lombardy. From its discovery on, several researches have been carried out to evaluate the best control agents against this pest. During field surveys in September 2016 and 2017 we realized that a discrete number of *P. japonica* larvae was infected by larval stages of a mermithid nematode. After collection, we reared in laboratory condition some specimens of this mermithid nematode to obtain adults necessary for the identification. Upon morphological and molecular analyses we concluded that the mermithid nematode was a new species that we described as *Hexamermis popilliae* (Poinar, 2017), drawing inspiration by the host in which we did the discovery. The life cycle of *H. popilliae* appears to be similar to that of other terrestrial mermithids. After completing its development in the host, the mermithid emerges as a third stage postparasitic juvenile. This stage molts twice (usually both cuticles are shed simultaneously) to the adult stage. Mating and oviposition occur in the soil. The preparasitic juveniles emerging from the eggs actively search for host larvae and enter the body cavity by direct penetration through the cuticle. This is the first record of an Hexamermis species parasitizing the Japanese beetle *Popillia japonica.* The only previous mention of mermithid nematodes associated to *P. japonica* was an undescribed species in North America belonging to the genus Psammomermis. Preliminary observations on the incidence of *H. popilliae* parasitization are shown. Further researches are necessary to evaluate if *H. popilliae* could be used in an IPM program for controlling the Japanese beetle in Italy, in association with the already used entomopathogenic nematodes.

Keywords: Popillia, Hexamermis, EPN, mermitid

PO186

FIELD SURVEYS ON NATIVE EGG-PARASITOIDS ATTACKING THE EXOTIC *HALYOMORPHA HALYS* IN NW ITALY

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The brown marmorated stink bug *Halyomorpha halys* (Hemiptera: Pentatomidae) is a native species to East Asia, which is now widespread in North America and Europe. It is a highly invasive harmful pest, responsible for serious damage on several crops. Chemical control is difficult to apply also due to the high pest mobility. Therefore, in the new invaded areas, research has been focused to identify native natural enemies able to attack *H. halys*, and evaluate their effectiveness as biological control agents. In NW Italy, where *H. halys* has been reported from 2013, field surveys were carried out in 2016-2017 to assess the recruitment of native egg parasitoids by *H. halys*. From mid-June to early September, egg masses of *H. halys* were periodically collected in 10 sites and transferred to laboratory, where they were reared until emergence of bug nymphs or parasitoid adults. In the two-year surveys, 1,107 egg masses (for a total of 28,915 eggs) were collected, of which 317 were parasitised, with a total parasitism rate of 12.4%. Four parasitoid species were obtained, among which *Anastatus bifasciatus* (Hymenoptera: Eupelmidae) was the most abundant. This generalist egg parasitoid emerged from eggs collected in all sites and in both years, with a parasitim rate variable from 1.0% to 19.6%. Moreover, one *Trissolcus* sp., one *Telenomus* sp. (Hymenoptera: Scelionidae) and one hyperparasitoid (Hymenoptera: Pteromalidae) were occasionally obtained. In particular, *Trissolcus* sp. emerged only from eggs collected in one site in both years, and its ability to develop on *H. halys* eggs was evaluated in no-choice laboratory trials aimed at assessing its potential as a candidate for biological control.

Keywords: Brown marmorated stink bug, biological control, parasitism rate, *Anastatus bifasciatus*

PO187

EVALUATION OF BT BAIT EFFICIENCY ON THE TURNIP (WINTER) MOTH *AGROTIS SEGETUM*

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The turnip moth, *Agrotis segetum* is considered as one of the most important pests on sugar beet. The larvae feed on young leaves and injure the root crown causing a relatively large hole in the crown of the plant. In this study, the efficacy of Bt Semisolid bait was compared with an organophosphate pesticide, Dursban in Azarbaijan- Gharbi and Lorestan provinces. Four treatments including Dursban (Chlorpyrifos) EC 40.8% 2l/ha, Bt bait (5l Bt (Biolop)+50l water+100 kg wheat bran)/ha, Bt (Biolop) Spray (3 ml/l), Semisolid Bt bait (100 Kg/ha), were used on the turnip moth in sugar beet fields in a randomized complete block design with four replications, in two periods, seven and 10 days after emergence. Analysis of variance, showed a significant reduction in damage rate on sugar beet while using Bt semisolid bait compared to other treatments in Azarbaijan-Gharbi. In the Lorestan province, the efficacy of Semisolid Bt bait and Dursban worked better than the other treatments.

Keywords: *Agrotis segetum*, Bt bait, Semisolid, Dursban (Chlorpyrifos), sugar beet

PO188

STUDY ON BIOLOGICAL CHARACTERISTICS OF SUNN PEST EGG PARASITOIDS

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Species composition of sunn pest egg parasitoids is related to region and canvas, but in a specified range of the time and place, one or a few species are more abundant. In order to evaluate and compare the life table parameters of sunn pest egg parasitoids, parasitized eggs were collected during sunn pest oviposition period in Qazvin province cereal fields and transferred to the laboratory of sunn pest research department. Then, the emerged wasps were reared on the sunn pests or Graphosoma eggs separately. Afterwards, the biological characteristics of each species including immature developmental period, adults' survivorship, fecundity, and longevity were measured. The faunistic studies results showed that there were two species of parasitoids including *Trissolcus grandis* and *T. semisttriatus* on sunn pest eggs in Qazvin province. Data analysis showed that immature developmental period of *T. grandis* was significantly lower than that of *T. semistriatus*. Moreover, the comparative studies of population growth parameters of *T. grandis* and *T. semistriatus* showed that net and gross reproductive rates of *T. grandis* were higher than those of *T. semistriatus* and totally, *T. grandis* had higher reproductive potential.

Keywords: Demography, Egg parasitoid wasps, Sunn pest, *Trissolcus grandis*, *Trissolcus semistriatus*

PO189

SOME BIOLOGICAL PARAMETERS OF THE *ORIUS VICINUS* RIBAUT (HEMIPTERA: ANTHOCORIDAE) AT THREE DIFFERENT TEMPERATURES

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Orius species are often recognized important biological control agents which are specifically used to control pest thrips (Thysanoptera) species. In this study, some biological features of *Orius vicinus* Ribaut (Hemiptera: Anthocoridae) on the eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) in the laboratory at three constant temperatures (20°C±1, 25±1°C and 30±1°C), 60±10 RH and 16L:8D photoperiod were investigated. At least 100 eggs laid into bean pods during 24 hours were isolated to the plastic cups (0.5 l capacity). Newly emerged first-instar nymphs were transferred to the plastic containers (5×2 cm) with help of a fine brush, and mortality, developmental time and sex ratios of the nymphs were determined. Following the adult emergence, preoviposition, oviposition and postoviposition periods were investigated. Additionally, adult longevity and mortality were also calculated. The nymphs and adults were fed upon *E. kuehniella* eggs, which had been exposed to U.V. radiation for the sterilization of the eggs and served as a part of bean pods for moisture source and oviposition substrate. Statistically significant differences were found for each nymphal stage. When the temperature increased, developmental time reduced for all immature stages. Duration of eggs hatching were calculated as 7.72±0.11, 5.37±0.06 and 3.61±0.06 days at 20°C±1, 25±1°C and 30±1°C, respectively. Duration of total nymph development were recorded 24.79±0.14, 13.81±0.09 and 9.93±0.08 days at 20°C±1, 25±1°C and 30±1°C, respectively. Furhermore, preoviposition, oviposition, postovposition periods, longevity and the numbers of eggs laid were statistically different. Duration of preoviposition, oviposition and postoviposition periods were the longest at 20°C±1. Although, there was a significant differences between the oviposition periods, the eggs numbers laid by a female was not different between 20°C±1 (139.42±12.56 eggs per female) and 30±1°C (142.45±10.44 eggs per female). The number of eggs laid was the highest at 25°C±1 (190.39±13.37 eggs per female). The longevity of females were recorded as 64.69±3.52, 44.88±2.22 and 27.70±1.06 days at 20°C±1, 25±1°C and 30±1°C, respectively. It is concluded that 25°C±1 temperature is favorable in means of rearing of this predatory insect.

Keywords: *Orius vicinus*, biology, temperature

PO190

INTRAGUILD PREDATION BY THE GENERALIST PREDATOR *ORIUS ALBIDIPENNIS* ON WHITEFLY *BEMISIA TABACI* PARASITIZED BY *ERETMOCERUS MUNDUS* AND *ERETMOCERUS EREMICUS*

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Intraguild predation of an oligophagous predator, *Orius albidipennis* Reuter, on two parasitoids of *Bemisia tabaci* Gennadius including *Eretmocerus mundus* Mercet and *Eretmocerus eremicus* Rose and Zolnerowichis was studied under laboratory condition. In choice test, adults and fifth instar nypmhs of *O. albidipennis* were exposed to second and third instar nymphs of *B. tabaci*, and larval and pupal stages of both parasitoids, separately and prey consumption was quantified. In choice test, prey preference of two stages of *O. albidipennis* for parasitized and unparasitized whitefly nymphs was studied when different combinations of preys offered to predators. The results showed that both unparasitized and parasitized nymphs of *B. tabaci* were easily preyed by two predator stages. However, both adults and fifth instar nymphs of *O. albidipennis* preyed most on *E. eremicus* larvae. Predation of predator stages was lowest on unparasitized nymphs of *B. tabaci* and *E. mundus* pupae. In all prey combinations, both adults and fifth instars of *O. albidipennis* had no clear preference for parasitised or unparasitised whitefly nymphs. According to the results, the presence of *O. albidipennis* may have a negative influence on parasitism rate of both *E. mundus* and *E. eremicus* on *B. tabaci*.

Keywords: Anthocoridae, Aphelinidae, Biological Control, Parasitoid, Predator

PO191

IDENTIFICATION OF THE MAIN COMPONENTS OF *TORYMUS SINENSIS* VENOM BY AN INTEGRATED TRANSCRIPTOMIC AND PROTEOMIC APPROACH

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Torymus sinensis Kamijo (Hymenoptera: Torymidae) is a hymenopteran wasp utilized for the biological control of the Asian chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), a globally invasive pest of chestnut, *Castanea* spp. *T. sinensis* is a univoltine ectoparasitoid, sometimes exhibiting a prolonged diapause, with a life cycle perfectly synchronized with its host. It is the dominant parasitoid species reared from *D. kuriphilus* galls in its native China, and has been introduced into many countries of Asia, North America, and Europe for the management of the gall wasp populations. We used high-throughput nucleic acid sequencing methods to describe the transcriptome of *T. sinensis* venom gland. From *de novo* assembly of all cDNA 22874 contigs were obtained, all sequences were analysed using Blast2GO software, a bioinformatic "tool" used for the annotation and functional analysis of genes or protein sequences. The transcriptomic information provided an overall picture of the putative proteins present in the venom gland and information on molecular functions, biological processes and putative cellular compartments. The proteomic analysis was carried out on the components of the venom, fractionated by SDS-PAGE electrophoresis. Protein bands were excised from the gel and, after tryptic digestion, were identified by mass spectrometry (MALDI TOF/TOF and LC-MS/MS). The comparison between the protein sequences identified using the MASCOT software and the transcriptomic data, allowed to identify numerous putative proteins of *T. sinensis* venom. Molecular identification and subsequent characterization of these molecules will be essential to understand the role played by venom in the induction and regulation of the pathological syndrome observed in parasitized hosts.

Keywords: *Torymus sinensis*, venom gland, RNAseq, proteomic analysis

PO192

BIOLOGICAL CONTROL OF FALSE CODLING MOTH, *THAUMATOTIBIA LEUCOTRETA*, USING ENTOMOPATHOGENIC NEMATODES

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False codling moth (FCM), *Thaumatotibia leucotreta*, is a priority pest of citrus, stone fruit and table grapes, as it causes direct crop damage. In South Africa, FCM has developed resistance to the majority of insecticides; additionally export markets now enforce lower chemical residues, encouraging the use of more environmentally friendly control methods. Currently, information on the alternate management of FCM on stone fruit and table grapes is lacking. Biological control has not been explored for use against FCM in stone fruit and table grapes. However, entomopathogenic nematodes (EPNs) have been shown to provide control against the larvae and pupae of FCM in laboratory bioassays. Currently, only Cryptonem (*Heterorhabditis bacteriophora*) is registered against the soil borne stages of FCM. EPNs are particularly attractive for the control of FCM, as they attack soil stages (*i.e.* larvae/pupae). In this study, multiple local EPN species and strains were tested in laboratory bioassays against FCM larvae, pupae and eggs. *Steinernema jeffreyense*, *in vitro*-reared at Stellenbosch University, was further tested in the field, by means of applying infective juveniles (IJs) to the vineyard floor. Four different concentrations (0, 10, 20, 30 IJs/cm^2) were applied to forty 1 m2 experimental plots that were artificially infested with FCM larvae. The insects were retrieved from the soil 48 h later, to allow for assessment of the immediate effect after exposure to the biological. In the laboratory, the EPNs were found to provide high control against the larvae, partial control of the pupae, and the first record of virulence against the eggs. The field study showed promising results, as the immediate effect of the nematodes caused up to 77% mortality of FCM larvae. EPNs should prove to be a valuable addition to the current integrated pest management programme.

Keywords: Local EPNs, environmentally friendly control, field trial, IPM

PO193

BEHAVIORAL RESPONSES OF *COCCINELLA SEPTEMPUNCTATA* AND *DIAERETIELLA RAPAE* TOWARD SEMIOCHEMICALS AND PLANT EXTRACTS

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The chemical ecology of natural enemies can play a pivotal role in any Integrated Pest Management (IPM) program. Different chemical cues help to correspond in diversity of associations between prey and host plant species. Recent studies exhibited that the use of natural enemy is an ecofriendly measure to control pests. *Coccinella septempunctata* and *Diaeretiella rapae* have the abilities to explore several chemical cues released by plants under herbivore attack that may enhance their efficiency of foraging. In this study, the behavioural responses of *Coccinella septempunctata* and *Diaeretiella rapae* were examined under the application of two semiochemicals and a plant extract and their combinations using four arm olfectometer. The bioassay was consists of a pairwise treatment comparison. Data pertaining to preference of *C. septempunctata* and *D. rapae* after treatment application were recorded and analysed statistically. The mean number of entries and time spent of *Coccinella septempunctata* and *D. rapae* were greater in arms treated with E-β-Farnesene. However, the efficacy of E-β-Farnesene was enhanced when combined with β-pinene. Thus, the mean number of entries and time spent of *C. septempunctata* and *D. rapae* were highest in arms treated with the combination of E-β-Farnesenexβ-pinene as compared with other treatments. The current work has demonstrated that the insect derived semiochemicals may enhance the efficacy of natural enemies when applied in combination.

Keywords: β-pinene, E β-Farnesene, olfectometer, bioassay

PO194

EVALUATION OF ENTOMOPATHOGENIC FUNGI AND THEIR CRUDE EXTRACT AGAINST PEACH FRUIT FLY, *BACTROCERA ZONATA*

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Infectivity of twenty one different isolates of entomopathogenic fungi was evaluated against last instar larvae and adult of fruit fly species *Bactrocera zonata* (Saunders) (Tephritidae: Diptera) under set environmental conditions. During screening bioassay all tested isolates at dose rate of 1×10^9 conidia ml-1 caused varying levels of mortality, but in general adult stage was found more susceptible compared with larvae. Five potential isolates selected from screening bioassay belonging to *Beauveria bassiana* and *Metarhizium anisopliae* were further evaluated at four different concentrations (1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia ml-1) against both stages. Again adults were more susceptible than larvae at all dose rates. During the assessment of ability to transmit the disease from infected to healthy adults it was found that significant mortality among different combinations was caused either male or female adults were infected. The application of crude extract from the most effective fungal isolate was also found to be highly effective against adult flies with significant mortality and reduced adult survival. This study indicates that fungal isolates have ability to be used in the integrated pest management of *B. zonata* and their crude extract may offer an additional incentive to be used in a bait spray to kill the adult flies.

Keywords: *Beauveria bassiana*, *Metarhizium anisopliae*, screening, crude extract, fruit fly, horizontal transmission

Session 4.
Chemical Ecology and Multitrophic Interactions

Invasive Alien Species, Population Genetics, Infochemical Network

PO195

SEXUAL BLUNDERS AND THE SLOW NORTH AMERICAN SPREAD OF A EUROPEAN TETROPIUM BEETLE

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Tetropium fuscum is a European longhorn beetle, native to Western Europe and Northern Eurasia, that invaded Halifax, Nova Scotia, Canada circa 1990. In the 25-30 years since its introduction into North America, *T. fuscum* has spread less than 100 km from its point of introduction. Contrasted with the invasive Emerald Ash Borer, which also invaded North America from Asia around 1990, the North American range expansion of *T. fuscum* is less than 10% that of Emerald Ash Borer. We propose that the slow North American spread of *T. fuscum* is at least partially due to interactions with the native congener *Tetropium cinnamopterum*. *T. fuscum* and *T. cinnamopterum* are morphologically and ecologically very similar, sharing closely timed emergence in the spring, as well as similar host plant choices and mating behaviours. One of the most intriguing similarities is that the male-produced mating and aggregation pheromone blends in both species share the molecule fuscumol as the attractive component. We ask whether mate choice errors and mating disruption could be mechanisms contributing to the slow North American spread of *T. fuscum*. Preliminary data show that both *T. fuscum* and *T. cinnamopterum* males will mate with heterospecific females under no-choice mating conditions, but that they do so at a significantly lower rate than with conspecific females. Furthermore, in choice mating experiments, both species of male beetle attempt to mate with heterospecific females even in the presence of a conspecific female, suggesting that mating errors may take place in the field. The presence of a second female had no significant effect on duration of copulation in mating pairs, regardless of whether the female engaged in copula was heterospecific or conspecific to the male. Our results support mate choice error as a possible contributing factor to the slow North American spread of *T. fuscum*.

Keywords: Invasion Ecology, Hybridization, Mate Choice, Mating Errors, Forest Entomology

PO196

THE ROLE OF VOLATILE ORGANIC COMPOUNDS (VOCS) IN MEDIATING THE HOST PLANT PREFERENCE OF *BAGRADA HILARIS*

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The Painted bug *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae) is an invasive stink bug species that attacks mainly cruciferous vegetables crops, wild mustards and several ornamental plants of *Brassica*ceae family. This pest has its origin in Asia and Africa, and recently has been reported in United States, Mexico and Chile. *Bagrada hilaris* is very aggressive to young seedlings of *Brassica* spp. and can cause severe tissue damage and plant mortality. The role of the volatile organic compounds (VOCs) emitted from plant that might elicit host preference of this pest was not yet been investigated. In this study the role of VOCs in the host preference of *B. hilaris* adults to three *Brassica* species at seedling stage (7-days old): *B. oleracea* var botrytis, *B. napus* and *B. carinata* was evaluated in arena and olfactometer bioassays. In arena bioassays, *B. hilaris* individuals showed preference for *B. oleracea* var botrytis and *B. napus* rather than form *B. carinata*. Proportionally, the feeding injury was higher on *B. oleracea* and *B. napus* seedlings than on *B. carinata*. Similarly, in olfactometer the *B. hilaris* adults showed preference for *B. oleracea* var botrytis and *B. napus* rather than for *B. carinata*. Preliminary chemical analysis on the VOCs profile of these three species indicate similarities on the main chemicals emitted from *B. oleracea* var botrytis and *B. napus* and evident differences with the VOCs emitted from *B. carinata*. These results seems suggesting the possible role role of olfaction in this host location processes of *B. hilaris*.

Keywords: *Bagrada hilaris*, *Brassica oleracea*, *Brassica napus*, *Brassica carinata*, Olfactometer, VOCs

PO197

CUES REGULATING *PHILAENUS SPUMARIUS* BEHAVIOUR

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The meadow spittlebug *Philaenus spumarius* L. (Hemiptera, Aphrophoridae) is the main vector of *Xylella fastidiosa* strain associated with the “Olive Quick Decline Syndrome” in South Italy. This insect is the most abundant species on non-olive vegetation in olive orchards as well as on olive foliage. It is extremely polyphagous reaching up to 158 species of food-plants. Nymphal stages were mainly associated to different herbaceous plants (*i.e.* Asteraceae, Fabaceae, Umbelliferae) whereas the adults shows a host shifting during the season. They moved from grass cover to woody plants, including olive trees, in summer and moved back to grass cover in autumn to oviposit. Little is know about *P. spumarius* ecology (*e.g.* feeding behaviour and host preference) and the mechanism underlying the shifting from herbaceous to woody plants. We studied *P. spumarius* behaviour and the chemical cues involved in these processes. The attractiveness of the *P. spumarius* adults towards two olive plant cultivars was tested in Y-tube olfactometer using plants *versus* clean air as odour sources, and testing adults (♂,♀) collected in the field either as juvenile stages or adults (sweeping). Juveniles were reared on barley and broad to reach the adult stage to be tested. Data presented here shown that females are more responsive than males and are more attracted by olive plants. Moreover, adults collected by sweeping were more responsive than those obtained from juvenile stages. Yet, there is a little difference for the attractiveness among olive cultivars. After each biossasay, volatile emitted by plants were analysed to study the cues and the mechanism underlying the insect behaviour. The data presented give the opportunity to identify compounds useful to divert pest communication and host plant location, and to develop selective traps for monitoring and mass-trapping.

Keywords: *Philaenus spumarius*, *Xylella fastidiosa*, CoDiRO, Olive trees

PO198

IDENTIFICATION OF VOLATILES RELEASED BY FRUIT-ASSOCIATED YEASTS FOR THE BIOCONTROL OF *DROSOPHILA SUZUKII*

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Chemical control of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) using synthetic insecticides is particularly challenging as it is difficult to respect pre-harvest intervals. Therefore, a different approach to tackle the problems caused by *D. suzukii* shall be developed. Applications within chemical ecology allow us to do this, modifying the insect's behaviour in the field. With this research, we aim to develop a new control strategy that will reduce or eliminate the residues of chemical insecticides on fruits, using targeting yeasts to attract specifically the oviposing females of *D. suzukii*. We cultured eight yeast strains, previously found to be fruit-associated, on two different media (Yeast Minimal Medium and Potato Dextrose Broth) to determine which volatile compounds (VOCs) they emit. The volatiles profile emitted by each living yeast culture was characterised using direct headspace analysis and gas chromatography-mass spectrometry (DHS-GC-MS). Results showed that *Saccharomyces cerevisiae* S288C release the highest emission of alcohols and ethyl esters, while the strains *Candida* sp. 3.3 and *Hanseniaspora* uvarum 1.21 release much more acetates and almost no alcohols. The strain *Saccharomycopsis* vini 1.23 instead, showed a high emission of just one acetate and some alcohols, but no esters. Electroantennography was then utilized to test the selected volatiles on the *D. suzukii* antenna. The ultimate aim will be to find a right blend of volatiles to create a formulation potentially applicable in the field.
Our research was kindly supported by the European EFRE project "Dromytal".

Keywords: Pest insects, yeasts, volatile compounds, field, fruit, chemical ecology

PO199

BEHAVIOR OF *STENOMA CATENIFER* IN ORCHARDS OF AVOCADO VARIETIES

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Stenoma catenifer, the avocado seed moth, is a new world species that is thought to feed exclusively on the fruit and seeds of plants in the family Lauraceae. It has been recorded feeding on fruit of avocados (*Persea americana*) and the greenheart tree, *Chlorocardium rodiei*, an important timber tree in Guyana. *Stenoma catenifer* is a well known avocado pest attacking fruit in Mexico, Guatemala, Costa Rica, Panama, Bolivia, Colombia, Peru, Ecuador, Venezuela, and Brazil. The objective was to evaluate the behavior of *S. catenifer* in avocado orchards in Brazil in three areas containing different varieties. Surveys of Hass avocados collected from commercial orchards in Brazil have revealed that 1-2 larvae per seed is the most common level of infestation. However, it is not uncommon to find the occasional seed with 3-4 *S. catenifer* larvae, and occasionally 7-8 larvae may be found feeding in one seed. In Brazil >55% of avocado fruit that are attacked by *S. catenifer* are in the upper half of the tree.

Keywords: Avocado seed moth, Elachistidae, Pest

PO200

POPULATION GENETIC STRUCTURE AND SYMBIONTS OF WHITEFLY *TRIALEURODES VAPORARIORUM* (HEMIPTERA: ALEYRODIDAE), IN THE UK

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Whiteflies are significant pests of many crops worldwide. The genetic diversity, population structure, and endosymbiosis identification of whitefly species have been studied in different regions, but there is a little knowledge about *T. vaporariorum* in the UK. The objectives of this study were to use mtCOI sequencing, and microsatellite approaches to investigate the genetic diversity and population structure of glasshouse whitefly *T. vaporariorum* collected across the UK as well as identify the symbiotic bacteria associated with it. This study tries to answer questions about the occurrence of haplotypes in the UK, the level of genetic differentiation among and within populations, symbiotic bacteria linked with this species and how habitat and agricultural management affect the genetic diversity and population genetic structure of this pest. The findings may help to improve the management of glasshouse whitefly in the UK.

Keywords: Genetic diversity, microsatellite, mitochondrial haplotype, mtCOI, population structure, endosymbionts.

PO201

ELECTRO-PHYSIOLOGICAL RESPONSES AND BEHAVIOR OF POTENTIAL BIOLOGICAL CONTROL AGENTS OF *HALYOMORPHA HALYS*

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The brown marmorated stink bug (BMSB) *Halyomorpha halys* has in recent years become one of the most damaging alien pests worldwide. Originating from Eastern Asia, it has spread throughout much of the United States, as well as into Canada. In Europe, BMSB was first detected in 2007 in Zurich, Switzerland; its range has now expanded to include most of the European and Eurasian countries. Currently, effective control relies on the use of broad spectrum insecticides that are disruptive to natural enemies and to already established IPM programs. The employment of egg-parasitoids is a promising tool for the sustainable management of BMSB, since they suppress the host in its early developmental stages before it feeds and inflicts crop damage. Understanding the behavior of native egg-parasitoids that have been recorded from *H. halys* in Europe, and unraveling the mechanisms by which they exploit chemical cues for host location are key in refining the biological control of this pest. We extracted volatiles belonging to males, females, nymphs and eggs of BMSB. Employing a coupled gas chromatography-electroantennographic detection (GC-EAD), the electrophysiological responses of two potential biological control agents, *Anastatus bifasciatus* and *Ooencyrtus telenomicida* have been measured. Identification of compounds eliciting antennal responses, establishment of dose-response curves and Y-tube olfactometer bioassays are being conducted to uncover whether these native parasitoids are attracted or repelled by BMSB volatiles. Compounds exhibiting kairomonal properties will be assessed for possible implementation in the enhancement of parasitoid foraging capacity and parasitization efficiency.

Keywords: Parasitoid, Chemical ecology, Insect behavior, GC-EAD, Pentatomidae

PO202

OLFACTORY RESPONSE OF PREDATORY BUG *ORIUS ALBIDIPENNIS* AND PARASITOID WASPS *ERETMOCERUS MUNDUS* AND *ERETMOCERUS EREMICUS* TO *BEMISIA TABACI* AND COMPETITOR NATURAL ENEMIES

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Cotton whitefly, *Bemisia tabaci* (Gennadius), is one of the most important pests of field crops, vegetables, and ornamental plants. Parasitoid wasps, *Eretmocerus mundus* Mercet and *Eretmocerus eremicus* Rose and Zolnerowich, and predatory bug *Orius albidipennis* (Rueter) are among natural enemies which are used as biological control agents of *B. tabaci* and Play a pivotal role in reduction of chemical insecticide usage. In this study, experiments were carried out using Y-tube olfactometer to determine the impact of odors produced by *B. tabaci* infested leaves of cucumber on attraction of natural enemies. The effect of presence of one natural enemy on attraction or repellency of other natural enemies was also studied. Results from olfactory experiments of synomone production by *B. tabaci* infected plants showed that infected cucumber leaves attract all three species of natural enemies including *O. albidipennis*, *E. mundus* and *E. eremicus* more than uninfected leaves. The results of the effect of parasitoid presence on the preference and selection of *O. albidipennis* showed that the bug avoids leaves containing both species of parasitoid wasps. However, presence of *O. albidipennis* on leaves as a competitor dose not disturb the parasitism behavior of parasitoid species.

Keywords: Aphelinidae, Anthocoridae, Olfactory, Synomone

Plant-Microbe-Insect Interactions

PO203

EFFECT OF WATERMELON SILVER MOTTLE VIRUS INFECTION ON THE FEEDING BEHAVIOR OF *THRIPS PALMI*

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The majority of plant viruses are exclusively transmitted by insect vectors. Virus may manipulate the behavior of its vector to increase its transmission rate which is known as vector manipulation hypothesis. Watermelon silver mottle virus (WSMoV) is transmitted by *Thrips palmi* and causes a serious problem in melon production. The objectives of this study was to examine the direct and indirect effects of WSMoV infection on the feeding behavior of *T. palmi*. The probing events of thrips were monitored by electrical penetration graphs (EPG). The probing events of the viruliferous and non-viruliferous thrips were compared, and significant direct effects of WSMoV infection were identified on the probing frequency for both females and males. The numbers of short ingestion probes and all probes of the viruliferous thrips were higher than those of the non-viruliferous thrips. The durations of non-ingestion, short ingestion, and long ingestion probes of the examined thrips were not affected by the virus infection. The indirect effects of WSMoV infection were examined by comparing the probing events of the non-viruliferous thirps feeding on the WSMoV-infected and healthy plants. The plant-mediated indirect effects on the probing behavior were different between sexes. The numbers of non-ingestion probes, short ingestion probes, and all probes were lower when the female thrips fed on the virus-infected plants than on the healthy plants. The duration of short ingestion probes was longer when the female thrips fed on the virus-infected plants, and so was total ingestion time per hour. Increasing short ingestion probes of the viruliferous thrips may benefit the virus to facilitate its transmission, and longer ingestion time of female thrips feeding on the WSMoV-infected plants suggests female thrips prefer to settle and feed on the infected plants. The results suggest WSMoV may manipulate the feeding behavior of *T. palmi*.

Keywords: Behavior, electrical penetration graph, Orthotospovirus, probing, thrips

PO204

TRICHODERMA HARZIANUM MODIFIES TOMATO TRANSCRIPTOME AND METABOLOME WHILE PROMOTING DIRECT AND INDIRECT DEFENSES RESPONSES

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Plant Growth Promoting Fungi belonging to the genus *Trichoderma* are among the most widespread soil microbes, which have been used as biocontrol agents of plant pathogens. Despite several reports about their promotion of plant defenses against pathogens are available, few information have been addressed to the enhancement of plant responses against insects. The possibility to develop improved formulations based on *Trichoderma* strains active against pests is a very attractive perspective for sustainable agriculture. A better understanding of the molecular mechanisms occurring during strain-cultivar interaction may help to achieve this objective. Here we investigate on the transcriptomic profiles following *Trichoderma harzianum* strain T22 treatment of the tomato cultivar “San Marzano nano” and subsequent aphid infestation. A wide transcriptome reprogramming related to metabolic process, regulation of gene expression and defense responses was observed. Early and late genes involved in direct defenses against insects are induced (*i.e.* peroxidase, GST, kinases and polyphenol oxidase, miraculin, chitinase). As far as genes involved in indirect defenses, sesquiterpene synthase and geranyl geranyl phosphate synthase are up-regulated. Targeted and untargeted semi-polar metabolome analysis revealed a wide metabolome alteration showing in treated plants an increased accumulation of isoprenoids, known defense-related compounds. These data nicely correlate to the observed increased attractiveness of Tricoderma treated plants towards the aphid parasitoid *Aphidius ervi*. In addition, in *Trichoderma*-treated plants we registered the over-expression of transcripts coding for several families of defense-related transcription factors (bZIP, MYB, NAC, AP2-ERF, WRKY) suggesting that the fungus contributes to prime plant responses against insect pests. Summarizing, our data indicate that *Trichoderma* treatment of tomato plants induces transcriptomic and metabolomic alterations resulting in a primed state of indirect defenses while showing a probable increase in direct defense responses.

Keywords: San Marzano, aphid, parasitoids, RNAseq, semi-polar metabolome

PO205

OVIPOSITION PATTERN OF *CAMERARIA OHRIDELLA* AND ITS CONSEQUENCES FOR OFFSPRING – INTERACTION WITH LEAF BLOTCH DISEASE AND LIGHT CONDITIONS

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Invasion of the horse chestnut leaf miner (HCLM, *Cameraria ohridella* Deschka & Dimić 1986) considerably changed the condition of its main host, *Aesculus hippocastanum*. After few centuries from introduction of this tree in Europe, HCLM achieved great ecological success by colonising whole continent in only 30 years. Spectrum of factors responsible for it is broad, but the intensification of transport, low environmental resistance and small number of competitors are most prominent. Although, the processes of the HCLM population dynamics and suitability with other host-plant species are well recognized, lower attention was paid for the ecological aspects of interaction with biotic and abiotic factors. Looking deeper into ecology of HCLM we conducted few *in vivo* experiments to test “mother-knows-best” hypothesis. Focusing on the interaction with leaf pathogen (*Guignardia aesculi*) and light conditions, the oviposition pattern and performance features of the HCLM were investigated. We analysed the egg deposition frequency taking into account leaf characteristics. Survival rate, development time, pupal mass and efficiency of food consumption were measured and used as indicators of the insect performance. Phytochemical measures complemented scope of the study. The egg frequency was highly correlated with the leaflet biometry rather than factors being under experimental control: presence of leaf blotch or light conditions. The co-occurrence of leaf phytopathogen had no distinct influence on the insect performance. In turn, the light intensity substantially changed chemical as well as structural characteristics of the leaves and whereby insect performance was affected. However, the summer active:diapausing pupae ratio was not under control of tested environmental factors. In view of our results, HCLM mothers deposit eggs not randomly, but offspring allocation is far away from the best choice. Mismatch between preference and performance may be explained by the risk minimization phenomenon.

Keywords: Ascomycota, insect performance, phytochemistry, leaf biometry, leaf-miner, Lepidoptera

PO206

WIREWORMS UNDER THE SWORD OF DAMOCLES: ROOT VOLATILE EMISSIONS EXPLAIN CONTRASTING FIELD AND LABORATORY ATTRACTION IN FOUR MAIZE VARIETIES

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Volatiles Organic Compound (VOCs) are one of the many features of defense used by plants in their eternal fight against pests. Their main role is to attract the natural enemies of the herbivores. But on another hand, they can be used by the same herbivores to locate plants while foraging. In an attempt to fill a gap of knowledge in a complex web of interactions, we focused on wireworms (Coleoptera:Elateridae). Wireworms whose larvae feed on roots are one of the most spread pests of valuable crops such as maize and potatoes, causing important economical damage. Little is known about the root compounds that are playing a role in the attraction of the larvae. In order to know more about these compounds, we compared four different maize varieties (*Zea mays mays*) that are known to have different levels of attraction, from weak to strong, for wireworms in fields. We collected, separated, identified and quantified the VOCs from the roots of all four varieties and showed qualitative and quantitative differences among the VOC identified. Finally, we performed laboratory bioassays where wireworms were offered all possible combinations of the four maize varieties. The choice of the larvae was clearly dependent on the offered combination and some varieties were preferred to others. Our aim is to develop traps based on VOCs from maize roots to open a new frontier in wireworms management.

Keywords: Direct defense, Integrated pest management, Maize roots, Volatiles organic compounds, Wireworms

PO207

RICE OSABA8OX3 MUTANT REVEALS A MECHANISM LINKING RESISTANCE TO *NILAPARVATA LUGENS* WITH INDUCED CALLOSE DEPOSITION

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Abscisic acid (ABA) is an important plant hormone that plays a significant role in regulating rice growth and development and in responses to drought, salinity, low temperature and other abiotic stresses. Prior studies had supported the hypotheses that ABA could affect the self-regulation of rice in the face of biological adversities such as fungi, viruses and pests and that the OsABA8ox3 gene played very important role in the ABA hydrolysis pathway of rice. In this paper, an experiment was performed to examine the resistance of the OsABA8ox3 T-DNA insertion mutant to *Nilaparvata lugens* Stål (Hemiptera: Delphacidae; BPH). The electrical penetration graph (EPG) results showed that rice resistance to BPH in the OsABA8ox3 mutant was stronger than that in the wild type (WT). After being fed by BPH, the OsABA8ox3 mutant showed a higher number of penetrations in the N1 wave and less total feeding duration in the N4 and N5 waves compared with WT. Further studies had shown that callose deposition was a key factor increasing rice resistance, and callose deposition in the OsABA8ox3 mutant was higher by 60.39%, 52.2%, 26.6% and 31.7% than that in WT after BPH feeding for 0, 24, 48, and 72 h, respectively. The relative expression of hydrolase genes in the OsABA8ox3 mutant was mostly lower than that in WT, while the relative expression of synthesis genes was mostly higher than that in WT. These results provided new insights into the mechanism of callose deposition regulation in response to piercing-sucking pest.

Keywords: OsABA8ox3 mutant, abscisic acid, rice, *Nilaparvata lugens*, callose

PO208

TESTING THE CO EVOLUTIONARY THEORY OF LEAF COLOURSORATION: INSIGHTS FROM THE CAATINGA DOMAIN

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Red colorization of senescing leaves has been linked to changes in the light/temperature relationship (photo-protection) and warning signaling for herbivores (coevolution). In the semiarid environment of Caatinga, seasoning is restricted to dry and wet periods and composes an ideal place for testing red coloration in deciduous trees without the influence of photo-protection theory. This study was conducted in the Caatinga reserve Fazenda Almas, in the Cariri of Paraíba, northerstern Brazil. Individuals of Spondias tuberosa had two twigs sampled each and were separated by color and type of damage. The damaged area of the leave was visually estimated and placed into four categories: no damage, from to 25%; 25 to 50% and above 50% of leave's area lost. Data were subjected to a Generalized Linear Model with a binomial distribution. Red leaves are more frequently damaged by pathogens and defoliators than green leaves, but miners damage was observed only in green leaves (X^2=55.9; P<0.001). GLM indicates that red leaves were more consumed in all the evaluated damage area: from to 25% (P<0.01); 25-50% (P<0.05) and above 50% (P<0.001). Globally, red leaves were more damaged than green leaves. Coevolution theory of red leaves suggests that the increase in the concentration of anthocyanin in the leaves is an effective defense against herbivores. However, our results contradicts such literature, by pointing that the red leaves are susceptible to either herbivore or pathogenic agents in this endemic species. Thus, red coloration as a warning signal is not supported for Spondias tuberosa in the tropic.

Keywords: Co-evolutionary theory, deciduous trees, tropics.

PO209

OLFACTORY RESPONSES OF GRAVID FEMALE AFRICAN ARMYWORM, *SPODOPTERA EXEMPTA* WALKER (LEPIDOPTERA: NUCTUIDAE) TO HOST PLANT VOLATILES

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African armyworm, *Spodoptera exempta* is one of the major migrant insect pests of cultivated cereal crops and grassland in sub-Saharan Africa, Arabian and Asian countries. The current uses of chemicals in the management of *S. exempta* have not given the desired result, hence the continuous huge yield losses yearly in endemic areas. The use chemical ecology principles are in vogue in the management of insect pests because of its environmental friendly disposition and effectiveness even in polyphagous insects. Unfortunately, this new technology has not been tried on this dreaded pest of economic crops. Hence in this study, we evaluated olfactory responses of gravid females to rice plant volatiles with a view to determine its potential in behaviour modification and management of the pest. The electrophysiological results showed that *S. exempta* responded significantly to the following chemical constituents of the host plant: 2-methyl heptanes, 3-Hexen-1-ol acetate,4-Hexen-1-ol acetate, B-Ocimene, Trans-sabinene hydrate, 1,2-Dimethoxybenzene (veratrole), Ethyl benzoate, 4-Ethlbenzaldehyde, Indole, Trans-B-Ionone 4-(2,6,6-Trimethyl-1-cyclohexenyl)-3-buten-2-one, Curcumene and alpha-murolene. These signify serious ecological and pest control implications. However, the nature of these responses have not been fully ascertained as the research is still ongoing. Our tentative conclusion therefore is that *S. exempta* responded significantly to host plant volatiles and could be harness in host location, pest monitoring, behaviour modification and control of the pest with possible substantial yield/economic returns. We recommend that more research be carried to determine the nature of these responses for better understanding and effect management of the pest.

Keywords: Olfactory Responses, host plant volatiles, African armyworm, *Spodoptera exempta*, pest management

PO210

DEVELOPMENT OF A 'LURE-AND-KILL' SYSTEM TO MANAGE BEETLE PESTS OF FIELD BEANS AND PEAS

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The pea and bean weevil (*Sitona lineatus*) and bruchid beetle (*Bruchus rufimanus*) are economically important agricultural pests of legumes, of global significance, that can lead to reduced crop yield and quality. Current pest management methods rely on blanket sprays of insecticide to the crop canopy, which is difficult to penetrate and often makes targeted insecticide applications challenging. Besides the practical difficulties of insecticide application, there is increasing concern regarding the effects of insecticides on non-target species and resistance. Consequently, there is a demand to improve insecticide targeting and develop practical alternatives to conventional insecticides. We are currently developing a pest management system that utilises semiochemical attractants to lure the beetles to a bait station containing a bioinsecticide in a collaborative project. The benefits of such a system to growers include: increased pest control through a more targeted application of the insecticide; reduced use of insecticide per hectare; the option of different modes of killing agent which allows the product to be available to both conventional and organic growers; and as large volumes of water are not be required for uniform application, this product can be applied during periods of water shortages. Laboratory based experiments have focussed on optimising the attractants and determining the effectiveness of two entomopathogenic fungi (*Beauveria bassiana*) isolates as bioinsecticides. Formulations were optimised for controlled emission of the attractants and efficacy of the entomopathogenic fungi as a bioinsecticide. Field based experiments have focused on establishing the efficacy of the lure and kill system compared to the conventional insecticide α-cypermethrin. Further field experiments are being undertaken during spring 2018. If successful this project will provide a new management solution for beetle pests in legume crops, which may be transferable to other pests in a variety of cropping systems.

Keywords: Semiochemical, Entomopathogen, IPM, Coleopteran Pests

PO211

APHIS GOSSYPII MODULATES DIRECT AND INDIRECT DEFENCE RESPONSES DURING A COMPATIBLE INTERACTION WITH *CUCURBITA PEPO* L. PLANTS

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Plants respond to aphid attack by reprogramming their transcriptome with the consequent synthesis of metabolites involved in direct and indirect defence. Zucchini is widely cultivated in temperate regions where the damage imposed by the cotton/melon aphid *Aphis gossypii* (Homoptera: Aphididae) represents a threat that can highly affect host plant yield. To identify genes involved in the molecular plant response to aphid feeding, we analysed the transcriptome of zucchini leaves at three time points (24, 48 and 96 h) following infestation with 10 *A. gossypii*. More than 900 genes were differentially expressed in aphid challenged *vs* control plants. The main GO categories influenced during the infestation were "cellular metabolic process", "primary metabolic process" and "response to stress". Among the genes associated with defence response we observed, on one hand, the up-regulation of enzymes involved in cell wall modification, SA biosynthesis and mediated response and, on the other hand, the down-regulation of enzymes involved in JA-mediated response. Genes associated with the attraction of natural enemies were all down-regulated and were mainly involved in the biosynthesis of secondary metabolites such as phenylpropanoids and terpenoids. The analysis of VOCs released by infested plants showed a significant modulation of (E)-caryophyllene emission depending on the magnitude of infestation: a reduction of (E)-caryophyllene was observed for plants infested for 48 h with 10 aphids, while plants infested with 300 aphids released a higher amount of (E)-caryophyllene. Olfactometer bioassays revealed that females of *Aphidius colemani*, a parasitoid of A.gossypii, are attracted by (E)-caryophyllene. These results suggest that the activation of plant indirect defence may be dependent on the severity of infestation. It appears that, with low infestation levels, aphids can supress defence and emission of VOCs involved in tritrophic interactions, however, if aphid numbers build up (E)-caryophyllene emission can no longer be supressed and the "cry for help" signal is released.

Keywords: Zucchini, RNA-seq, aphid, gene expression, VOCs

Molecular Basis of Insect Communication

PO212

THE LARVAL ECTOPARASITOID HOLEPYRIS SYLVANIDIS RECOGNISES SUITABLE HOSTS BY THEIR CUTICULAR HYDROCARBONS

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Many parasitic wasps are known to use cuticular hydrocarbons (CHCs) of their insect hosts as contact kairomones for host finding and recognition. Our previous studies showed that the larval ectoparasitoid *Holepyris sylvanidis* orients along host larval CHC traces to its preferred host, *Tribolium confusum*. Furthermore, host recognition assays revealed that *H. sylvanidis* recognizes *T. confusum* as host by the larval CHC pattern [1]. So far, it is unknown whether CHCs of other potential host beetle larvae also serve *H. sylvanidis* as contact kairomones for host recognition. Therefore, we (a) studied the behavioural response of *H. sylvanidis* toward various potential host species and their CHCs, and (b) chemically analyzed the CHC patterns of these host species and searched for CHC patterns characteristic for preferred host species. In contact bioassays the parasitoid showed typical host recognition behaviour when encountering dead and live larvae of Tribolium species (*T. castaneum*, *T. confusum*, *T. destructor*). In contrast, larvae of *Oryzaephilus surinamensis* were rejected. The parasitoid recognised the latter species only after extracting dead larvae with n-hexane and applying *T. confusum* larval CHC extract. GC-MS analysis of larval extracts showed that CHC profiles of the three Tribolium species were almost identical, whereas the CHC pattern of *O. surinamensis* larvae differed qualitatively and quantitatively from the patterns of the Tribolium species. Our data indicate that the rejection of *O. surinamensis* larvae is due to the lack of certain (groups of) CHCs. We are currently testing fractions of the active larval extracts to identify the compounds relevant to host recognition. Based on our findings, *H. sylvanidis* represents a promising candidate for biological control of various Tribolium species.

Reference

[1] Fürstenau & Hilker 2017. Journal of Chemical Ecology 43:858-868.

PO213

BEHAVIORAL RESPONSE OF *ORIUS LAEVIGATUS* (FIEBER) AND *ORIUS MINUTUS* (L.) TO THREE THRIPS-ATTRACTION CHEMICALS IN A Y-TUBE OLFACTOMETER

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Orius laevigatus (Fieber) (Hemiptera: Anthocoridae) and *Orius minutus* (L.) are important natural enemies of various small arthropods such as aphids, thrips, whiteflies, and mites in agricultural ecosystem. Chemical attractants of both insect pest and its natural enemies can be used to improve the efficacy of biological control through push-pull strategy. We investigated the response of the two *Orius* spp. to attraction chemicals of *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), *i.e.*, methyl isonicotinate, (1S)-(−)-verbenone, and aggregation pheromone, in Y-tube olfactometer. Higher proportion of *O. laevigatus* (mated unfed adult females) moved to the arm of methyl isonicotinate or (1S)-(−)-verbenone than the arm of clean air. But, given-up rate was lower in methyl isonicotinate than (1S)-(−)-verbenone. Nevertheless, O. minutus did not show any significant preference to the chemicals tested. These results suggest that, among the three attractants, methyl isonicotinate would be a good attractant for both *O. laevigatus* and thrips.

Keywords: biological control, push-pull strategy, minute pirate bug, methyl isonicotinate, verbenone, pheromone

PO214

ENLARGING KNOWLEDGE ON CANTHARIDIN IN BLISTER BEETLES (COLEOPTERA: MELOIDAE)

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Cantharidin (CA) is a toxic terpenoid produced by blister beetles, transferred from males to females during copulation. In addition, as a characteristic defensive behaviour, blister beetles can exudate CA-containing hemolymph by reflex bleeding from leg joints. Despite attracting the interest of many researchers for its properties (CA is a promising compound for many medical applications), some basic knowledge enlightening the role of CA in blister beetles' ecology are still lacking. For instance, data on CA content in many meloid species are still unavailable and the quantification of CA in the exuded hemolymph, despite its renowned defensive role, has been completely ignored. The objective of this study was to quantify the CA content in the exuded hemolymph (to be compared with CA quantity in body tissues) of eight species never investigated so far, belonging to Mylabris, Lydus, Meloe and Cerocoma genera. Specimens were collected in Italy, identified and reared in fauna boxes. The exuded hemolymph from each specimen was collected from leg joints every two days using capillary tubes and stored at -70°C. Insects body tissues were also stored at -70°C for subsequent analyses. Available protocols were optimized for CA extraction from both body tissues and exuded hemolymph and CA content quantified in GC-MS using a calibration curve with an internal standard. CA content in the exuded hemolymph resulted highly variable among species (from ~ 100 ng/mg in *Cerocoma schreberi* to ~2800 ng/mg in *Mylabris fabricii*) and typically less concentrated than in insect bodies. Interestingly, CA content in insect tissues greatly varied among individuals collected from a single locality (*e.g. Lydus trimaculatus* and *Mylabris variabilis*), with, apparently, no correlation with sex. The implications of our findings are discussed and future perspective to outline the chemical diversity and ecological role of CA and related-compounds are presented.

Keywords: Cantharidin, Blister beetles, Reflex bleeding, Defensive behaviour

PO215

LARGE EXPANSION OF GUSTATORY RECEPTORS IN THE GENOME OF THE NOCTUID MOTH *SPODOPTERA LITTORALIS*

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The evolution of chemosensory receptors is expected to play an important role in the adaptation of insects to a diversity of ecological niches. Whereas the function and the evolution of olfactory receptors have been the focus of intense research in the last years, the gustatory receptors (GRs) have received less attention. GRs are expressed in taste organs and are believed to detect non-volatile molecules such as sugars and bitter compounds found on food sources and oviposition sites. We annotated the repertoire of candidate GRs in the genome of *Spodoptera littoralis*, a polyphagous noctuid moth. We found a massive expansion of GRs, with more than 200 genes annotated. Most of these genes were found in large gene clusters, suggesting recent and repeated tandem duplications. Phylogenetic analyses revealed that this expansion occurred in candidate bitter receptor clades, and is not restricted to *S. littoralis* as a large number of candidate bitter receptor genes arranged in clusters could also be identified in closely related species. GR expansion could thus constitute a hallmark of Noctuidae, the most diversified family of Lepidoptera. In order to investigate expression patterns of GR genes, we also carried out a transcriptomic study on different taste organs of *S. littoralis*, and compared expression patterns with those observed in other lepidopteran species. This work lays the foundation for future studies on the link between the evolution of GRs and the adaptation to polyphagy in Noctuidae.

Keywords: Chemical communication, gustatory receptor, Lepidoptera, *Spodoptera littoralis*

PO216

INVESTIGATIONS ON MOLECULAR MECHANISMS AND MORPHOLOGY OF STRUCTURES INVOLVED IN CHEMORECEPTION IN BLACK SOLDIER FLY LARVAE *HERMETIA ILLUCENS* (DIPTERA: STRATIOMYDAE)

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Insects use chemical perception to interact with other insects and with their environment. The perception of volatile substances, linked to the processes of feeding, mating and escape from predators, is related to gene families encoding for proteins belonging to Odorant Binding Proteins (OBPs), Olfactory Receptors (ORs), Ionotropic Receptors (IRs) and Chemosensory Proteins (CSPs). The larval stages of the black soldier fly *Hermetia illucens* L. (Diptera: Stratiomyidae) are generalist detritivores which feed on decomposing organic materials of both animal and vegetal origin. *Hermetia illucens* has a highly sensitive olfactory system, specialized in perceiving molecules linked to the decomposition of organic materials. Investigations on molecular mechanisms involved in feeding source recognition mediated by OBPs, through the perception of volatile attractants and the study of the structures involved in olfactory perception may contribute to the description of ethological details of this species (*e.g.* acceptance or rejection of organic material by larvae). An identification of the protein profile has been conducted based on the generation and annotation of a *H. illucens* larval *de novo* transcriptome and subsequent identification of transcripts encoding proteins involved in processes mediating olfactory perception. The OBPs and other predicted proteins involved in olfactory perception mechanisms were identified using BLAST searches and protein domain signatures. A parallel investigation by scanning electron microscopy (SEM) has been conducted on *H. illucens* larvae to identify structures possibly related to the olfactory perception. The preliminary data obtained by the two experimental approaches will be useful to increase knowledge about physiology of this insect.

Keywords: Black soldier fly, chemoreception, RNA-seq, scanning eletron microscopy

PO217

BLACK SOLDIER FLY TRANSCRIPTOME ANALYSIS RELATED TO THE IDENTIFICATION OF CANDIDATE CHEMORECEPTIVE PROTEINS FOR THE DETECTION OF EGG VOCS

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In insects, the recognition of volatile organic compounds (VOCs), involved in the processes of feeding, mating and escaping from predators, is mediated by proteins belonging to the families of Odorant Binding Proteins (OBPs), Olfactory Receptors (ORs), Ionotropic Receptors (IRs) and Chemosensory Proteins (CSPs). In order to select optimal oviposition sites, adult black soldier flies, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), are attracted not only to organic materials but also to the presence of eggs laid by conspecific females. To identify chemoreception candidate transcripts, RNA-seq was performed on *Hermetia illucens* antennae and whole body of male and female adults. The *de novo* transcriptome assembly resulted in 78,763 contigs and functional annotation using Blast2GO identified 59 putative OBPs, 186 ORs, 56 IRs, 7 CSPs. VOCs sampling was conducted in order to identify volatile organic compounds emitted by black soldier fly eggs. These were collected about six hours after oviposition; approximately 3 g of eggs were partitioned into equal units and subsequently into equal replicates. VOCs sampling, through a Closed Loop Stripping Analysis (CLSA) method, was performed on each unit and replicates directly at initiation (Day0) and 24h (Day1), 48h (Day2), 72h (Day3) after oviposition, respectively. The identification of attractive volatile organic compounds (VOCs), linked to the functional characterization of proteins involved in chemoreception, will allow the understanding about molecules perceived by females in the oviposition process and provide indications and guidelines for designing and screening new attractive substances, analogous to the identified compounds, with the aim to improve insect mass production.

Keywords: Black soldier fly, RNA-seq, chemoreception, egg VOCs

PO218

STUDY OF THE MOLECULAR MECHANISMS AND MORPHOLOGICAL ANALYSIS OF STRUCTURES INVOLVED IN CHEMORECEPTION IN *CAPNODIS TENEBRIONIS* (COLEOPTERA, BUPRESTIDAE)

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Insects use chemical perception to interact with biotic and abiotic environmental factors. The perception of volatiles is mediated by molecules belonging to gene families, namely Olfactory Receptors (ORs), Ionotropic Receptors (IRs), Chemosensory Proteins (CSPs) and Odorant Binding Proteins (OBPs). *Capnodis tenebrionis* (Coleoptera, Buprestidae) infests mainly apricot, cherry, peach and plum. It colonizes the wide geographical area of the Mediterranean. The adult consumes bark and buds while larvae dig subcortical galleries in root cambium and phloem, compromising the vegetative and productive activity of the plant. The control of this pest lacks of monitoring strategies, such as information on insect chemical ecology. The composition of perceived volatile organic compounds (VOCs), as well as their emission rates, play an important role in determining the signal specificity for *C. tenebrionis* repulsion or attraction. The VOCs emission from control plants and from drought elicited plants was investigated through off-line (GC-MS) and online (PTR-MS) spectrometric techniques. The identification of the gene expression profile was carried out through an "omics" approach, based on RNA-seq, *de novo* construction and annotation of male and female adults transcriptome, using Blast2GO and searching for transcripts coding for proteins involved in the olfactory perception processes. Subsequently, OBPs and other transcripts putatively involved in olfactory perception were identified using BLAST searches and protein domain signatures. To investigate the putative roles of chemosensilla involved in the selection of the host plant, scanning electron microscopy (SEM) was performed on male and female *C. tenebrionis* individual antennomers. Chaetica, basiconica and coeloconica sensilla were recognized on the antennae. *Coeloconica multipore sensilla* were often found in pits on the paraxial and antiaxial faces of V-XI flagellomers.

Keywords: *Capnodis tenebrionis*, chemoreception, RNA-seq, VOCs, scanning electron microscopy

Session 5.
Ecology and Toxicology of Insecticides

Insecticide Resistance

PO219

SUB-LETHAL EFFECT OF PHOSALONE AND AMITRAZ ON CELLULAR ENERGY ALLOCATION (CEA) IN THE COMMON PISTACHIO PSYLLID, *AGONOSCENA PISTACIAE*

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The cellular energy allocation (CEA) methodology is used to evaluate the effects of abiotic stress on the energy metabolism of organisms. The purpose of this study was to determine the effects of phosalone and amitraz pesticides on CEA in the common pistachio psyllid, *Agonoscena pistaciae*, one of the most important of pistachio pests, that was resistant to these pesticides more than 7-fold. The energy reserve fractions (Ea) including total lipid, sugar, glycogen and protein were measured in the *A. pistaciae* that were treated at LC30 and LC50 concentrations of amitraz and phosalone, before treatment as well as 4 and 8 hours after the treatment. The results indicated that significant difference between the amounts of Ea in different concentration of tow pesticides. The amount of lipid affected more that other fractions by different concentration of phosalone and the amount of lipid in LC10 and LC50 concentration was significantly increase than the control after 8h of exposure. Results showed that energy consumption (Ec), was significantly different ($P<0.05$) between phosalone and amitraz in this population. The amount of CEA affected by different concentration of amitraz and the amount of CEA in LC50 concentration was significantly increase than the control after 4h of exposure. It appears that the CEA methodology may be use an indicator for distinguish the sub-lethal effect of pesticides.

Keywords: *Agonoscena pistaciae*, Cellular Energy Allocation, Energy Consumption, Pesticide Resistance, Energy Reserve

PO220

A NOVEL WIND TUNNEL ASSAY FOR EVALUATING THE BEHAVIOUR OF PYRETHROID RESISTANT *AEDES AEGYPTI* FROM AREAS OF SAUDI ARABIA

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Aedes aegypti is the main mosquito that transmits dengue fever in Saudi Arabia. Typically, vector control tools using insecticides and insecticide-treated bednets (ITNs) can reduce disease-causing mosquito populations. Two cities endemic for Dengue, Makkah and Jeddah, have mosquitoes that are highly resistant to the pyrethroid, deltamethrin. We have shown that target site (kdr mutation) and metabolic mechanisms of insecticide resistance can negatively impact vector control strategies. To date, neither the efficacy of ITNs in Saudi Arabia, nor mosquito responses to the host odour through the holed nets is known. In this study, we assessed the ability of host-seeking *Ae. aegypti* (pyrethroid susceptible and resistant strains) to enter and survive passage through a hole in ITNs barrier. Field-collected insecticide resistant mosquitoes from Jeddah and Makkah, and a fully susceptible New Orleans strain were released individually to fly upwind towards holed nets (untreated/PermaNet® 2.0) in a wind tunnel. The behavioural events (Flying, Resting, Bouncing, Visiting) were digitally recorded for 20 min and analysed. The results showed that 100% Makkah, 87.5% Jeddah and 60% New Orleans mosquitoes successfully entered the holes towards a host odour in untreated net. In contrast, very few New Orleans (9% by chance) mosquitoes entered the holed PermaNet®2.0 compared to Makkah (58%) and Jeddah (36%). The more aggressive behavior of the insecticide resistant *Aedes aegypti* strains was statistically significant when compared to the susceptible strain. Makkah and Jeddah mosquitoes are also able to pass through holes in PermaNet® 2.0 quicker than New Orleans mosquitoes, and resistant mosquitoes survive passage at higher rates than susceptible. These data indicate that a PermaNet® 2.0 net might fail to protect against the resistant Makkah and Jeddah mosquitoes. However, further behavioural studies are needed to understand mosquito behaviour to ITNs alongside other vector control interventions.

PO221

BIONOMICS OF *ANOPHELES SERGENTII* AND FIRST MOLECULAR INVESTIGATION OF INSECTICIDE-RESISTANT GENES: KDR AND ACE-1, IN MOROCCO

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Anopheles sergentii is considered as the main vectors of the malaria in the south of Morocco. The disease was eliminated in 2008 and only imported cases are reported nowadays, but Morocco remains vulnerable because of the intensive circulation of gametocytes carriers, the lack of protective immunity of resident population and the presence of potential vectors in formerly malarious areas as well as the increasing risk of reemergence of malaria due to climatic and environmental modifications. This study provides baselines information on *An. sergentii* bionomics and for the first times its resistance to insecticides in Morocco by investigating the presence of Kdr L1014F and Ace-1 (G119S) mutations. *Anopheles sergentii* adults and larvaes were collected during the seasonal activity for 2 years. The environmental parameters of the breeding sites were recorded. The blood meal was analyzed using the PCR-RFLP method. Alongside the WHO routine susceptibility test with DDT (4%), malathion (5%), the *An. sergentii* specimens collected in the rural areas of Marrakesh city in Morocco were screened for the Kdr and ace-1 mutations. As results, *Anopheles sergentii* was found during summer and autumn seasons, feeding mainly on sheeps, cows and only 6,097% on human. The adults were collected indoors indicating predominantly endophilic behaviour. WHO bioassays revealed a complete susceptibility to DDT 4% and malathion 5%. Several variants were detected in the exonI and exonII of the voltage gated sodium channel, but no L1014F Kdr mutation was found. The PCR-RFLP revealed the absence of the ace-1 (G119S) mutation as well.

Keywords: Bionomics, blood meal, *Anopheles sergentii*, insecticide resistance, vector, Morocco

PO222

FIRST REPORT OF L1014F-KDR MUTATION IN *CULEX PIPIENS* COMPLEX FROM MOROCCO

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Mosquitoes of the *Culex pipiens* complex, competent vectors for West Nile virus and Rift Valley fever virus are widely targeted by insecticide treatments. The intensive application of chemical insecticides led to the development of resistance in many insects including *Culex pipiens* mosquitoes. The absence of data on resistance mechanisms in Morocco allow us to assess the levels of lambda-cyhalothrin resistance and the frequency of the mutated gene L1014F kdr in different forms of *Cx. pipiens* complex from three regions of Morocco. Mosquito adults were reared from immature stages collected in Tangier, Casablanca and Marrakech. Standard WHO insecticide susceptibility tests were conducted on adults emerged from collected larvae. Specimens were identified as belonging to the *Cx. pipiens* complex using a multiplex PCR assay with diagnostic primers designed from the flanking region of microsatellite CQ11. Identified mosquitoes were then tested for the presence of the L1014F kdr mutation using PCR assay. Our results showed that 21% of the tested population has a resistance to lambdacyhalothrin. The molecular identification of survivors shows that 43% belonged to the *Cx. pipiens pipiens* and only 9.5% to the *Cx. pipiens molestus* form. On the other hand, 416 specimens were screened for the L1014F kdr mutation. The frequency of L1014F mutation was similar between the pipiens form and hybrid form, while it was lower in the molestus form. The presence of the L1014F kdr allele was significantly associated with resistance to lambda-cyhalothrin in pipiens and hybrid forms. Resistance to lambda-cyhalothrin of *Cx. pipiens* populations appears to be largely due to this mutation. To our knowledge, the frequencies of L1014F kdr mutation are examined for the first time in natural populations of the *Culex pipiens* complex in Morocco. These findings will provide important information to propose more adapted vector control measures towards this mosquito species, potential vector of arboviruses.

Keywords: *Culex pipiens*, L1014F kdr, Lambda-cyhalothrin, Resistance, Morocco.

PO223

COMPARATIVE TRANSCRIPTOME ANALYSIS OF EMAMECTIN BENZOATE-RESISTANT AND MEVINPHOS-RESISTANT *PLUTELLA XYLOSTELLA*

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Diamondback moth (DBM, *Plutella xylostella* L.) is one of the main insect pests of cruciferous vegetables. It has developed insecticide resistance to almost all insecticide ingredients. To postpone or delay the development of insecticide resistance and to extend use of insecticide in *P. xylostella*, it is necessary to discover more insecticide-resistant mechanisms and Achilles' heel of resistant individual. The comparative transcriptome analysis of emamectin-resistant (Em-R)/emamectin-relaxed (Em-Rx) and mevinphos-resistant 96CM/mevinphos susceptible 96C pairs of DBM, which has no cross resistance to each other, have been accomplished. It is interested to find that the types, number and expression levels of cuticle proteins, esterases, solute carriers and ABC binding cassettes were extremely different in these two pairs of DBM. More cuticle proteins were expressed significantly higher in the Em-R stain than the 96CM strain, and the up- or down-regulated expression of cuticle proteins in these two sets of DBM were opposite. On the other hand, the esterases, solute carriers and ABC transporters were expressed significantly higher in the 96CM stain than the EM-R strain. These results imply that more decrease of penetration is involved in emamectin-resistance, while more detoxification and secretion are associated with mevinphos resistance. Therefore, cuticle proteins and esterases/solute carriers/ABC transporters can be the targets for investigating the Achilles' heel of emamectin-resistant and mevinphos-resistant DBM, respectively.

Keywords: *Plutella xylostella*, insecticide resistance, Achilles' heel, comparative transcriptome analysis, emamectin benzoate, mevinphos

PO224

GENETIC MECHANISMS OF RESISTANCE TO PHENYLPYRAZOLES IN THE BROWN PLANTHOPPER

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The brown planthopper (*Nilaparvata lugens*) is one of the most economically important rice pests in South and East Asia. The control of brown planthopper has relied heavily on synthetic insecticides of all the major classes, including organophosphates, carbamates, pyrethroids and neonicotinoids. Consequently, resistance to all of these classes of insecticides has evolved and is widespread across Asia. To circumvent this problem, in the last decade a different class of insecticide, the phenylpyrazoles, in particular ethiprole and fipronil, have been widely used in brown planthopper control. However, resistance to ethiprole and fipronil has also been reported in recent years. We investigated the molecular mechanisms of resistance to these compounds in the brown planthopper. Here we report the results of whole-genome DNA and RNA sequencing of susceptible, naturally resistant and artificially ethiprole-selected strains of brown planthopper combined with functional assays to identify genes associated with resistance to phenylpyrazoles.

PO225

FUNCTIONAL CHARACTERIZATION AND SIGNAL TRANSDUCTIONS ANALYSIS OF LATROPHILIN RECEPTOR IN *TRIBOLIUM CASTANEUM*

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Latrophilins (LPHs) are adhesion G-protein-coupled receptors comprising three paralogous forms (LPH-1, LPH-2 and LPH-3) and known receptors

for α-latrotoxin, which involved in growth, development, adaptability and schizophrenia and other diseases in vertebrates. However, the functions of LPH were poorly understood in most insects. Here, latrophilin (Tclph) was cloned in *Tribolium castaneum*, and three alternatively spliced transcripts (Tclpha, Tclphb and Tclphc) were identified. All these three Tclphs were highest expressed at the early adult stage, and strongly expressed in central nervous system of adults. Larval RNA interference (RNAi) against Tclph caused 24% adult wing abnormal, 30% insect death, and led to 100% reductions in beetle fecundity. Further, exon-specific RNAi illustrated that neither knockdown of Tclpha nor Tclphc led to development defects and reductions in beetle fecundity. Thus, it indicated that Tclphb is essential for development and female fecundity in *T. castaneum*. Furthermore, the global transcriptome profiles between RNAi treated larvae (ds-Tclph) and control larvae of *T. castaneum* were investigated and compared by using RNA-sequencing. Totally, 274 differentially expressed genes (DEGs) were identified, between the ds-Tclph and control samples. These DEGs were classified into 42 GO functional groups, including such functions as the immune system, the response to stimulus, insecticide metabolism, developmental and reproduction process. Interestingly, knock-down of Tclph suppressed both of Toll and IMD immunity pathways which most likely modulated the effects of Tclph on lifespan and stress resistance. Furthermore, RNAi and pesticides analysis of these differentially expressed genes were supported that Tclph is broadly involved into the developmental and insecticides detoxification in *T. castaneum*.

PO226

INVESTIGATION ON THE EFFECT OF IMIDACLOPRID DIFFERENT CONCENTRATIONS ON LIPID PEROXIDATION AND ANTIOXIDANT DEFENSE ENZYME ACTIVITY IN THE TERMITE *MICROCEROTERMES DIVERSUS* (ISOPTERA: TERMITIDAE)

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Microcerotermes diversus Silvestri is one of the most important pests of wooden and cellulosic products in residential areas, as well as in agricultural lands and green spaces in different parts of Iran, including Khouzestan province. Imidacloprid is a contact/ingestion pesticide that causes the death of an insect through disruption postsynaptic neuronal acetylcholine receptors. The aim of this study was to determine a biomarker by measuring the activity of antioxidant enzymes and the amount of lipid peroxidation in inducing oxidative stress in association with imidacloprid exposure. For this purpose, the effect of four different concentrations (5, 10, 25 and 50 ppm) of imidacloprid on important oxidative stress indices such as malondialdehyde (MDA) and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX) and glutathione reductase (GR) were studied in the worker caste. All treatments, including control, were performed in four replicates at 28±2°C and 85±5% relative humidity in a dark incubator. Results showed that the content of MDA and the activity of SOD and CAT enzymes in the termite hemolymph increased under the treatment of insecticide compared to control, but the activity of POX and GR enzymes decreased. Based on the results, imidacloprid resulted in oxidative stress in the insect and changes in the lipid peroxidation and antioxidant activity of the insect body.

Keywords: Malondialdehyde, Superoxide dismutase, Catalase, Peroxidase, Glutathione reductase, Ascorbate peroxidase

PO227

BASELINE SUSCEPTIBILITY AND ASSESSMENT OF RESISTANCE RISK TO FLUBENDIAMIDE IN *TUTA ABSOLUTA* (MEYRICK) (LEPIDOPTERA: GELECHIIDAE) POPULATIONS FROM KUWAIT

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Climate change and less stringent agricultural product import policies have facilitated the introduction of the invasive tomato leafminer *Tuta absoluta* (Meyrick) in Kuwait, and crop losses of up to 100% have been reported. *T. absoluta* has develop resistance and cross resistance to a wide range of insecticides in the field. Therefore, this study aimed to assess the baseline toxicity of field populations of *T. absoluta* to flubendiamide, a diamide insecticide recently registered to control the pest. Subsequently, the risk of resistance evolving, as well as inheritance and stability of resistance to flubendiamide were investigated. The susceptibility of 8 field populations to flubendiamide were determined through leaf dip bioassays, and the variation among the populations was low (3-fold), with LC50 values ranging from 0.04 to 0.11 mg L–1. A field population of *T. absoluta* was selected after repeated exposure to flubendimide to determine the risk of resistance evolution. After 24 generations of selection, there was a 70-fold increase in resistance to the pesticide. The realized heritability (h2) of resistance was estimated as 0.21 using threshold trait analysis. Resistance declined significantly within 8 generations in the absence of selection pressure, indicating that resistance to flubendemide was unstable. The results suggest that flubendiamide is highly effective against *T. absoluta*, and a solid baseline data has been established that can be used for future monitoring studies. However, there is a high risk of flubendiamide resistance in the field, and strategies for managing the evolution of resistance are recommended. The finding that resistance to flubendemide is unstable has significant practical implications for resistance management strategies that could be exploited. If resistance monitoring activities reveal that resistance to flubendemide has evolved in the field, removing the insecticide from spray schedules will allow the eventual reestablishment of susceptibility to the product, consequently, prolonging its efficacy.

Keywords: Tomato leafminer; flubendiamide; baseline toxicity; resistance; selection, reversion; resistance management

PO228

SUSCEPTIBLY AND RESISTANCE OF R-(+), S-(-)-LIMONENE AND CITRUS ESSENTIAL OILS AGAINST TWO COLOMBIAN STRAINS OF *AEDES AEGYPTI*

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Essential oils and their secondary metabolites are considered as new strategies to control mosquito larvae. The essential oils (EO) obtained from the peels of the citrus genus are a good source of limonene. The limonene has two isomers, R-(+)-limonene and S-(-)-limonene, which could be found on different proportions over the essential oils. These isomers and the EO's were evaluated in three different strains of *Aedes aegypti*, two Colombian field strains (Piedecuesta and Bucaramanga) and a lab strain (Rockefeller). For the determination of the lethal concentrations and resistance ratios, the WHO protocols were used. It was found that the S-(-)-limonene has a greater activity than the R-(+)-limonene whit an LC50 of 14.89 (13.88 -15.94) ppm and 22.77 (22.48-27.58) ppm respectively in the Piedecuesta strain; in the Bucaramanga strain the tendency was the same. The S-(-)-limonene showed an LC50 of 19.42 (17.99 – 21.42) ppm and the R-(+)-limonene of 25.66 (22.85 -28.77) ppm. Form the first approach to the evaluation of the susceptibly of these strains against the citrus essential oils (*C. sinensis*, *C. reticulata*, *C. tangelo* and *C. limon*), it was found that all the larvae tested died in concentrations over 80 ppm. Now, in terms of resistance, it was found that both wild strains are susceptible to the limonene isomers, meanwhile the Piedecuesta strain had showed resistance to malathion and chlorpyrifos with RR50 13.55 and 15.48 respectively. Is known that the organophosphorus insecticides are inhibitors of the enzyme acetylcholinesterase, in this case the limonene isomers and the citrus essential oils they could be consider as non-inhibitors of the enzyme whit IC50 between 149.8±7.9 and 285.9±24.5 ppm. The R-(+), S-(-)-limonene and the Citrus essential oils are new attractive ways to control different strains of *Aedes aegypti* due their low lethal concentrations, non-resistance and low toxicity over the enzyme acetylcholinesterase.

Keywords: Acetylcholinesterase, *Aedes aegypti*, Citrus essential oils, R-(+)-limonene, Resistance, S-(-)-limonene

PO229

SUSCEPTIBILITY OF CABBAGE STEM FLEA BEETLE (*PSYLLIODES CHRYSOCEPHALA* L.) TO PYRETHROIDS AND NEONICOTINOIDS V CZECH REPUBLIC

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The susceptibility of two local populations of cabbage stem flea beetle (CSFB) from Czechia to six pyrethroids, two neonicotinoids, one organophosphate and one oxadiazine was evaluated in 2015 - 2017 in a vial test according to IRAC no. 31, no. 21, no. 25 and no. 27, respectively. The LC50 values for the pyrethroids lambda-cyhalothrin, cypermethrin, esfenvalerate, tau-fluvalinate, etofenprox and deltamethrin ranged from 0.096 to 0.52 mg/L, from 1.54 to 6.20 mg/L, from 1.66 to 3.87 mg/L, from 1.95 to 21.8 mg/L, from 20.6 to 26.2 mg/L and from 0.046 to 0.58 mg/L, respectively. The LC50 values for chlorpyrifos, indoxacarb, and acetamiprid ranged from 3.47 mg/L to 168 mg/L, from 5.98 mg/L to 7.64 mg/L and from 0.42 mg/L to 7.43 mg/L, respectively. In both local populations, low susceptibility of CSFB to neonicotinoid thiacloprid was recorded with LC50 values ranging from 158 to 34,776 mg/L.Effect of seed dressing with thiamethoxam and thiacloprid on oilseed rape injuries caused by CSFB and Phyllotreta species was studied. The mortality of CSFB adults reached 100% on oilseed rape plants containing 8,860 mg/kg of thiamethoxam. The injury of plants was negligible. No mortality of CSFB adults was recorded on plants containing 9,960 mg/kg of thiacloprid and the injury was similar to oilseed rape plants without seed dressing. In small plot experiments, the neonicotinoid intake dynamics and degradation of their active substances relied on weather conditions. Seed dressing with thiamethoxam was highly efficient for control of oilseed rape plants against CSFB and Phyllotreta species. In contrast to this, stains based on thiacloprid are inefficient for control of oilseed rape plants due to high tolerance or resistance of Phyllotreta species to thiacloprid.
The work was funded by project QK1820081.

Keywords: Bioassay, resistence, susceptibility, seed dressing, *Psylliodes chrysocephala*, Phyllotreta

PO230

THE EFFICACY OF INSECTICIDES AGAINST DIAMONDBACK MOTH LARVAE FROM THE LITOMĚŘICE (CZ) REGION

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Aim of the study was to evaluate efficacies of different insecticides on a field population (Litoměřice) of *Plutella xylostella* (L.). Leaf dip method was used and the evaluation was done 48 hours after the treatment. Laboratory bioassays of the diamondback moth larvae (L2, L3) showed that the efficacies of some pyrethroids, especially deltamethrin, are insufficient. LC50 and LC90 values for deltamethrin were 341.4 and 36,504.7 ppm respectively. On the other hand, leaves treated with thiacloprid, cyantraniliprole, and Bt kurstaki caused 100% mortality in the diamondback moth larvae. Based on the results, the resistance of the field population of *Plutella xylostella* (L.) to deltamethrin was confirmed and this active ingredient should no longer be used in the region of Litoměřice. Furthermore, there are potential options among biological and synthetic insecticides, which should be used to control this pest.
The work was funded by projects No. QK1610217 and RO0418.

Keywords: Bt, cyantraniliprole, deltamethrin, efficacy, insecticide, *Plutella xylostella*, resistance, spinosad

PO231

BIOASSAYS - QUICK PROOF OF COLORADO POTATO BEETLE FIELD RESISTANCE

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The Colorado Potato Beetle (*Leptinotarsa decemlineata*) (CPB) is one of the most important potato pests worldwide. CPB has a remarkable ability to adjust to toxicants by developing a resistance. More than 300 cases of resistance to 56 insecticides are reported worldwide. Until the mid 1990s CPB resistance was monitored in Croatia and farmers were provided precise data about the status of specific insecticides. Implementation of insecticides with differing modes of action enabled farmers to overcome CPB resistance to organophosphates and pyrethorids. In Croatia recent failures in CPB control indicate that CPB might develop resistance to the newest generation of insecticides. Here we present results from bioassays carried out on CPB larvae with the aim to establish an inexpensive and reliable field resistance monitoring tool. In the bioassays performed, CPB are exposed to different doses of the insecticides and responses (mortality) recorded at the specific post-exposure interval. The mortality data are subjected to Log Dose probit analysis to generate estimates of a lethal dosage that provides mortality to 50% (LD50). When these data are collected for a range of the insecticide-susceptible populations, the LD50 can be used as baseline data for future monitoring purposes. After populations have been exposed to insecticides, the results can be compared to a previously determined LD50 levels using the same methodology. Such bioassays provide a reliable basis for initial characterization of resistant CPB populations, without the need of preserved specimens, expensive equipment or specialized expertise, but require many live specimens and lot of lab work. Despite the bioassays´ drawbacks, they are relatively simple, inexpensive and reliable method to assess resistance of field CPB populations and present a starting point for a more complete understanding of resistance including biochemical and molecular mechanisms that can be undertaken to ultimately build a resistance diagnostic toolbox for this global pest.

Keywords: Ootato, Colorado Potato Beetle, insecticide, resistance, LD50, bioassay

PO232

CEREAL APHID & BYDV CONTROL IN LIGHT OF RESISTANCE EMERGENCE

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Aphids are ubiquitous crop pests which can cause significant yield losses in economically important cereals. Cereal aphids are the most serious pests of cereal crops, they reduce yield and grain quality through direct feeding and virus vectoring. Barley yellow dwarf viruses (BYDVs) and the related Cereal yellow dwarf virus (CYDV) are a serious threat to cereals worldwide. The viruses produce leaf yellowing and stunting, leading to yield losses ranging from 5 to 80%, with an average of 30% in affected fields. Control of cereal aphids and the virus they vector, B/CYDV, is becoming increasingly difficult due to diminishing availability of effective control agents, development of insecticide resistance, the sustainable use directive and climate change. In Ireland the grain aphid, *Sitobion avenae*, is the most widely recorded species in the field and the main vector of the prevalent MAV strain of BYDV. Control has relied heavily on the application of pyrethroid insecticides. However, recent research in Ireland has confirmed the presence of aphids with the knock down resistance (kdr) mutation in field collected grain aphids, which confers varying resistance to pyrethorid insecticides. Aphids carrying the kdr mutation have been recovered from the major grain growing counties in Ireland. Trials are conducted on both low and high pressure sites in Ireland to validate the effectiveness of our current control advice in light of these challenges. Our trials aim to understand the implications of grain aphid resistance in Irish populations for management and efficacy of pyrethroid insecticides in controlling grain aphids and BYDV in crops, so that we have robust control options in place for Irish growers.

Keywords: Grain aphid, BYDV, knock down resistance, pesticide resistance, *Sitobion avenae*, IPM, Integrated Pest Management

PO233

Poster withdrawn.

PO234

THE ACTIVATION OF ABC TRANSPORTER GENES IN ADULTS OF THE MALARIA VECTOR *ANOPHELESI STEPHENSI*, IN RESPONSE TO PERMETHRIN

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The main tool for pest control is still represented by the use of massive doses of chemical insecticides, awfully pollutant and not always effective, due to the onset of insecticide-resistance forms. Therefore, investigating the defense mechanisms developed by arthropods against xenobiotics is an important research field towards the development of novel control strategies. In this work we focused our interests on the first line of cellular defense, the ABC transporters, membrane efflux pumps, that reduce the intracellular concentration of several toxic compounds. Their involvement in resistance outbreak has been underlined also against permethrin, the insecticide mostly used to control the Asiatic malaria vector, *Anopheles stephensi*. Starting from previous results obtained on larvae, our project aim was to investigate the defensive role of six ABC transporters against permethrin, in adult mosquitoes. Bioassays were performed separately on male and female of *An. stephensi*; both sexes were exposed to permethrin for one and 24 hours. At each time point mortality and gene expression of ABC transporters (AnstABCB2, AnstABCB3, AnstABCB4, AnstABCBmember6, AnstABCC11, and AnstABCG4) were analyzed and compared in the two sexes and at the two time points. We found a significant difference in mortality between male and female mosquitoes at both time points, that reflects the difference showed in gene-induction timing (more responsive in females than in males). Moreover, the expression comparison between the six genes showed that among three up-regulated genes in adults, two (AnstABCBmember6 and AnstABCG4) had previously also been shown to be overexpressed in larvae treated with permethrin. These results provide further evidence on the role of these genes in permethrin defense, during all developmental stages of *An. stephensi*.

Keywords: Vector-control, Mosquitoes, Detoxifying enzymes, Insecticides

PO235

SURVEY ON THE DISTRIBUTION OF TARGET SITE RESISTANCE TO PYRETHROIDS IN *VARROA DESTRUCTOR* IN LOMBARDIA (NORTHERN ITALY)

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Varroa destructor is one of the major pest for honey bees and one of the most important factors contributing to colony losses in many parts of the world. This emerged also from a recent survey among beekeepers in Lombardy region (Northern Italy). The aim of the present work was to diagnose the presence and distribution of target site resistance among V. destructor populations in this region as a part of a biennial project funded by the CARIPLO Fundation to evaluate the effects of different stressors on bee colonies. Resistance to tau-fluvalinate was first reported in Lombardy in 1991 but the specific evaluation of resistance mechanisms involved has never been carried out. For this reason, in summer 2017, *V. destructor* populations were sampled in different districts of this region. Mites’ DNA was extracted by “salting out” and the presence of the mutation L925V was monitored with an allel specific TaqMan assay. On the whole the survey was carried out on 74 bee colonies in 25 different localities and 286 specimens were analysed by real time PCR. The L925V mutation was detected but in low and variable ratio. Most of the specimens were “wild type”. Homozygous resistant were present in a ratio ranging from 2.7% up to 19.4% according to the districts. Just a couple of heterozygous specimens were identified. The presence of alternative polymorphisms producing mutations linked to resistance in USA (L925I and L925M) was investigated in randomly selected samples and in the heterozygous ones by direct sequencing, with negative results. This work confirms for the first time that resistance to pyrethroids in Northern Italy is linked to target site resistance nevertheless, according to this survey, the investigated mutation is not very widespread and tau-fluvalinate remains an useful alternative for the control of *V. destructor*.

Keywords: *Varroa destructor*, pyrethroids, target site resistance, L925V

PO236

DROSOPHILA MELANOGASTER AND ITS MICROBIOTA: THE RESPONSE OF THE HOLOBIONT TO XENOBIOTIC CHALLENGE

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Bioremediation exploits organisms' metabolic pathways, ensuring a low environmental impact and offering a valid alternative to traditional methods of decontamination. Even though bacteria and fungi are the major source of xenobiotic degrading enzymes, alternative organisms may permit to complement and optimize the metabolic pathway of degradation with novel molecules. Under this perspective, insects represent a potential and relatively unexplored alternative for detoxification of a wide variety of compounds due to their genetic plasticity and adaptability. Drosophila was selected as a model species for its proven ability to develop field resistance to the main insecticide classes. A resistant strain of *Drosophila melanogaster* (rD) was selected through continuous rearing on diet with 2000 ppm of pentachlorophenol (PCP), a biocide extensively used as wood preservative and in textile industries. In the last decades PCP has been considered a priority pollutant and its use has been restricted in several countries, due to its toxicity, long persistence, and bioaccumulation in fat tissues. Analyses of biological parameters (LD50, cycle length, weight) showed that the rD performs better than the wild type when reared on medium containing PCP. At 2000 ppm the mortality of the wild type was over 99%. HPLC analysis of contaminated medium where resistant larvae have fed showed a significant reduction of PCP content, compared to control. However, as the response of an organism to a xenobiotic might be the result of the interaction with its microbiota, attention was focused also on its associated microorganisms. Microbiota of resistant and susceptible *Drosophila melanogaster* produced statistically different DGGE patterns when compared. The putative resistance of bacteria from gut of rD larvae was also studied. From this preliminary results rD seems to be a potential new tool for xenobiotic detoxification.

Keywords: Bioremediation, DGGE, HPLC, Insect gut symbionts, Pentachlorophenol

PO237

DIAMIDE RESISTANCE IN LEPIDOPTERAN SPECIES AS CONFERRED BY TARGET-SITE MUTATIONS IN THE RYANODINE RECEPTOR

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Introduction: A notorious pest on cruciferous crops, the diamondback moth (*Plutella xylostella*) causes global economic damage, whilst resisting control by most insecticide classes. A breakthrough in the control of this lepidopteran pest came through the development of a new chemical class of insecticides, termed diamides, which act as conformation-sensitive activators of insect ryanodine receptors (RyR). However, resistance to diamides in Plutella has recently been reported in various geographical populations, each thought to be due to one or a combination of point mutations on the transmembrane domain of the RyR. *Methods:* An important first step to combatting the sudden increase in resistance is to investigate how these mutations are impacting the binding of diamides. The goal of this study is to set up an in-vitro system to screen for the effects of existing and predicted mutations on the RyR. The ability of the protein to bind radio-labelled ligands will be investigated in insect cell lines. Mutations on the protein, based on those reported in wild populations, will be introduced in order to examine their effect on ligand binding. *Results:* Initially, results will determine which trans-membrane residues have an impact upon the binding of diamide insecticides, prompting inferences on the molecular interactions within the proposed binding site complex of the protein in lepidopteran pests. In turn, this will aid understanding of RyR selectivity and diamide binding issues across various taxa.

Keywords: Lepidoptera, Plutella, Ion Channel, Diamide, Insecticide, Resistance, Crucifer

PO238

WESTERN CORN ROOTWORM ABUNDANCE, INJURY TO MAIZE, AND RESISTANCE TO CRY3BB1 IN THE LOCAL LANDSCAPE OF PREVIOUS PROBLEM FIELDS

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Western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is a major pest of maize in the Midwestern United States. Transgenic Bt maize is an important tool used to manage WCR populations and reduce yield loss. However, field-evolved resistance to all commercially available Bt toxins has been documented in the state of Iowa, USA. In areas where resistance is established, resistant individuals may travel from one field to another within a localized landscape, spreading resistance alleles. An important question that remains to be answered is the extent to which greater-than-expected root injury (*i.e.*, >1 node of injury) to Cry3Bb1 maize from WCR may affect the risk of WCR prevalence, root injury, and resistance in neighboring fields. To address this question, fields with a history of greater-than-expected injury to Cry3Bb1 maize (focal fields) and surrounding fields (<2.2 km from focal fields) were examined to quantify WCR abundance, root injury, and survival on Cry3Bb1 maize. Three years of field data and two years of bioassay data are presented with implications for management tactics in areas where WCR resistance to Bt maize has been identified.

Keywords: Western corn rootworm, Bt, transgenic, maize, resistance

PO239

SELECTIVITY OF PYRETHROID TO APHIDOPHAGOUS PREDATORS MEDIATED BY RESISTANCE TO INSECTICIDES

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The contribution of natural enemies for pest control is widely restrained by insecticides, especially under intensive use of broad-spectrum insecticides. Conservation of predatory insects regarding insecticide selectivity is sporadic considering that the reason of the synthesis and insecticide recommendations is to kill insects. Thus, the primary step to evade insecticide action is to evolve resistance to its inherent purpose. Thus, many insect species have becoming resistant to insecticides, especially herbivorous species. Sharing crop ecosystems with their prey, natural enemies are also exposed to insecticides and are selected to resistance. When exhibiting such trait, the natural enemy is able to tolerate efficacious doses of the insecticide recommended to the pest species. Therefore, physiologically the insecticide select its target's pest and not the natural enemy. This new attained selectivity through resistance has practical rationality when the outcome of pest control is additive or synergistic. It is possible when the insecticide and the natural enemy act on distinct pest species. The studied system consists of pyrethroid insecticides, which are efficacious against defoliating pests, but lacking efficacy against sucking-sap pests, such as aphids. Otherwise, lady beetles and lacewings are key predators of aphids and other sucking-sap pest species. Therefore, the expected outcome is at least additive. In this paper we will be presenting data with the neotropical lady beetle, *Eriopis connexa* resistant to the pyrethroid lambda-cyhalothrin and the control of diamondback moth, *Plutella xylostella*, an worldwide key pest of brassicas exhibiting also resistance to pyrethroids. Through caged trials, resistant *E. connexa* reduced to 5% survival of resistant DBM population. A mixed diet of aphid and DBM`s larvae was maintained by *E. connexa* even under high availability of the aphid, a preferred prey. Additional individual behavioral studies indicates that *E. connexa* does not present alteration in searching and predatory behavior under insecticide-treated environment.

Keywords: Coccinellidae, lambda-cyhalothrin, Insecticide selectivity, biological control

PO240

KETOENOL TOXICITY IN MEDITERRANEAN POPULATIONS OF THE WHITEFLY *BEMISIA TABACI*

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The whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) is among the most important pests worldwide. Here we monitored the efficacy of spirotetramat and spiromesifen, that belong to spirocyclic tetronic acid derivatives (ketoenols) to 15 *B. tabaci* MED populations from all over the Mediterranean Basin and their role in the neonicotinoid resistance management. Populations from Greece and Italy displayed the lower LC50 values, for both spirotetramat (LC50 <7 mg/L) and spiromesifen (LC50 <8.25 mg/L). Populations from Spain however, exhibited variable LC50s ranging from low (LC50=1.8 mg/L) to high values (LC50=250 mg/L) for spirotetramat and from low (LC50=1.4 mg/L) to extremely high values (LC50 >4000 mg/L) for spiromesifen. Although the correlation among the LC50s for ketoenols was not very strong, the higher LC50s for both ketoenols were exhibited by the same populations. In addition, among the populations that exhibited reduced susceptibility to imidacloprid none displayed reduced susceptibility to ketoenols, suggesting absence of cross resistance between the two groups of insecticides. Genetic studies using a highly resistant spiromesifen strain revealed that resistance was inherited autosomally and incompletely recessive. Results from F1 back-crosses suggested that resistance is polygenic. A next generation sequencing approach is followed to investigate resistance mechanisms by examining the differential expression between susceptible and resistance strains. Our results show the development of resistance in *B. tabaci* towards both ketoenols, which is geographically restricted to the South Spain. The use of ketoenols in alternation with insecticides with different mode of action classes as well as continuous monitoring of the efficacy should improve the sustainable chemical control of this major pest.

Keywords: *Bemisia tabaci*, ketoenols, insecticide resistance, resistance mechanisms RNAseq

PO241

PYRETHROID RESISTANCE IN THE OLIVE FRUIT FLY *BACTROCERA OLEAE*: EVOLUTION, MOLECULAR CHARACTERISATION AND OPERATIONAL IMPACT

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A reduction of pyrethroid (alpha cypermethrin) efficacy was recently recorded in some populations of the olive fly *Bactrocera oleae*, the most destructive

insect of olive trees, as indicated by an extensive monitoring survey in Greece. More specifically, a sharp increase in pyrethroid resistance was observed after 2013 in populations from the island of Crete, one of the most productive olive oil regions where pyrethroid insecticides have been used more frequently. The phenotype has an apparent impact on control efficacy, as demonstrated by field – cage trials, where the efficacy of standard bait application was shown to be significantly compromised. Sequence analysis of the IIS4–IIS6 region of para sodium channel gene in a large number of resistant flies indicated that resistance is most probably not associated with target site mutations, in line with previous studies in other Tephritidae species. We analyzed the transcriptomic differences between resistant populations *versus* a susceptible field population and laboratory strains. A large number of genes were found to be significantly differentially transcribed across pairwise comparisons. Interestingly, gene set analysis revealed that genes of the 'electron carrier activity' GO group were enriched in one specific comparison, which might suggest a P450-mediated resistance mechanism. The upregulation of several transcripts encoding detoxification enzymes was validated by qPCR focusing on transcripts coding for P450s. It is noteworthy that the expression of contig00326 and contig02103, encoding CYP6 P450s, was significantly higher in all resistant populations, compared to susceptible ones. These results suggest that an increase in the amount of the CYP6 P450s might be an important mechanism of pyrethroid resistance in *B. oleae*.

Keywords: Olive fly, pyrethroid resistance, P450s

PO242

MOLECULAR CLONING AND CHARACTERIZATION OF GABA RECEPTOR AND GLUCL GENES IN THE WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS*

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To understand the role of target site insensitivity in abamectin resistance in the western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), cDNAs encoding gamma-aminobutyric acid receptor subunit (FoRdl) and glutamate-gated chloride channel (FoGluCl) were cloned from WFT, and both single nucleotide polymorphisms (SNPs) and mRNA expression levels of FoRdl and FoGluCl were detected in a susceptible strain (ABA-S) and a laboratory selected strain (ABA-R) displaying 45.5-fold resistance to abamectin. Multiple cDNA sequence alignment revealed three alternative splicing variants of FoRdl and two alternative splicing variants of FoGluCl generated by alternative splicing of exon 3. While sequence comparison of FoRdl and FoGluCl in ABA-S and ABA-R strains identified no resistance-associated mutations, the expression level of FoGluCl in ABA-R strain was 2.63-fold higher than that in ABA-S strain. Thus, our preliminary results provide the evidence that the increased mRNA expression of FoGluCl could be an important factor in FoGluCl-mediated target site insensitivity in WFT.

Keywords: Rdl, GluCl, insecticide resistance, SNPs, *Frankliniella occidentalis*, abamectin

Insecticide Mode of Action: Biological and Biochemical Aspects

PO243

IPM STRATEGIES FOR WHITE FLY INSECTICIDES IN EUROPEAN GREENHOUSE OF TOMATO

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The presentation discusses the practical aspects of balancing efficacy against target and emerging pests, effective resistance management, maintenance of the beneficial insect populations and in addition sustainability of spray programs using predaceous arthropods in combination with chemical solutions, all in relation to seasonal control of white flies (*Bemisia* sp, *Trialeurodes* sp) on tomato. Reference is made to recent research and scientific literature which has enabled Dow AgroSciences to develop recommendations for the integration of the insecticide portfolio Closer (sulfoxaflor, Isoclast active), Flipper (fatty acid), spinosad (Laser) and spinetoram (Exalt) into IPM programs in tomato.

Keywords: IPM, *Bemisia* sp, white fly, insecticides, predators

PO244

EVALUATION OF INSECTICIDAL, BIOLOGICAL AND BIOCHEMICAL EFFECTS OF MONOTERPENES AGAINST THE COTTON LEAFWORM, *SPODOPTERA LITTORALIS* BOISD. (LEPIDOPTERA: NOCTUIDAE)

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Insecticidal, biological and biochemical activities of the three commercial oxygenated monoterpenes, 1,8-Cineole, Fenchone and Menthol, were evaluated against the cotton leafworm, *Spodoptera littoralis*, using different application methods. The ovicidal activity showed that the concentration of 4% of menthol achieved 100% mortality in treated eggs, while fenchone and 1,8-cineole recorded only 49.11% and 36.24%, respectively. Contact toxicity assay demonstrated that the most potent compound against *S. littoralis* larvae was menthol with LC50 value of 0.01 mg/cm^2, followed by fenchone and 1,8-cineole with LC50 values of 0.05 and 0.12 mg/cm^2, respectively. In fumigation assay, fenchone was more potent than 1,8-cineole against 4th instar larvae of *S. littoralis*. Values of LC50 were 2.7, 5.31 and <50 mg/cm^2 for fenchone, 1,8-cineole and menthol, respectively. Larval mortality percentages of the three compounds used as a residual film were 20.0, 20.0 and 31% for 1,8-cineole, fenchone, and menthol, respectively, compared with 4.0% for control. Menthol significantly decreased percentage of pupation to 70% compared with 96% for control. Menthol at a concentration of 2.5% caused 23.0 and 27.5% reduction in male and female pupal weights, respectively. Menthol significantly decreased percentage of adult emergence of *S. littoralis* to 54% compared with 88% for control, while adult emergence percentages were 63 and 65% for 1,8-cineole and fenchone, respectively. Mentohl caused higher antifeedant activity than fenchone and 1,8-cineole. Topical application assay demonstrated that 1,8-cineole, menthol and fenchone at a concentration of 50% caused percentages of pupation of 2.5, 17.5 and 35%, respectively. The most potent inhibitor of larval AChE activity was 1,8-Cineole with I50 value of 1.865 mM followed by Menthol and Fenchone with I50 values of 3.082 and 5.303 mM, respectively. Fenchone showed the strongest total proteases inhibition activity with I50 value of 1041.48 mM followed by Menthol and Cineol with I50 values of 1278.59 and 2254.14 mM, respectively.

Keywords: Monoterpenes; biology; AChE; total proteases; insecticides; *Spodoptera littoralis*

PO245

EFFECTS OF AZADIRACHTIN ON HEMOCYTE BEHAVIOR OF *GALLERIA MELLONELLA* (LEPIDOPTERA: PYRALIDAE)

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As a biodegradable natural insecticide, azadirachtin has attracted a great interest in integrated pest management programs. In this study, we investigated whether azadirachtin, as a good candidate for the integrated pest control, alters the cellular immune reactions of the model insect *Galleria mellonella* L. (Lepidoptera: Pyralidae). Different concentrations of azadirachtin (100, 1000, and 3000 ppm) was applied topically to the final instar larvae of *G. mellonella*. The number of mitotic, spread, and melanized hemocytes was assessed by counting the cells applied on a microscope slide after 24 h azadirachtin applications. The relative number of mitotic hemocytes decreased in a concentration-dependent manner, but the decline ratio was only significant at 1000 and 3000 ppm compared to untreated controls. The ratio of hemocytes showing spreading behavior significantly decreased at all azadirachtin concentrations with respect to untreated and distilled water treated groups. Azadirachtin markedly increased the hemocyte-mediated melanization at 1000 ppm and caused a decline at 100 and 3000 ppm according to untreated controls. When experimental groups compared with each other, the rate of melanized hemocytes was significantly lesser at 3000 ppm according to 1000 ppm. In nodulation experiments, control and topically azadirachtin-treated larvae after 24 h were injected with laminarin on the first hind leg. The darkened melanized nodules embedded in fat body, hemolymph and other organs were counted 24 h post laminarin injection. Larva treated with azadirachtin-laminarin gave a concentration-dependent decrease in the level of nodule formation. The reduction ratio of nodules rose to 76% at 3000 ppm compared to the laminarin-injected control. This study indicates that azadirachtin has potent effects on cellular immune reactions of *G. mellonella*.

Keywords: *Galleria mellonella*, azadirachtin, cell spreading, nodulation

PO246

INSECTICIDAL AND SYNERGISTIC EFFECTS OF MIXTURES OF CERTAIN SYSTEMIC AND CONTACT INSECTICIDES ON *MYZUS PERSICAE* (SULZER)

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The green peach aphid, *Myzus persicae* Sulzer, is one of the most important plant pests worldwide. Excessive use of chemical pesticides to control this pest has resulted in resistance of this insect to several groups of insecticides. One of the tactics to overcome or delay development of resistance is the use of mixtures of pesticides. In this research toxicity of pymetrozine, imidacloprid, deltamethrin and lambda-cyhalothrin on green peach aphid was initially assessed using leaf dipping method. The aphids were reared on bell pepper plants at 26±2°C, 60% RH and 16: 8 (L: D) photoperiod. All bioassays were scored 48 h after treatment, except for pymetrozine which was scored 96 h after treatment. The concentrations used in the initial bioassays were 200, 400 and 600 mg ai l-1 for deltamethrin and lambda-cyhalothrin; 25, 50 and 75 mg ai l-1 for imidacloprid, and 50, 100 and 200 mg ai l-1 for pymetrozine. At the concentrations tested, imidacloprid with mortalities of 55, 95 and 100%; and lambda-cyhalothrin with 100% mortality in all concentrations had the highest effect and were chosen for further experimentation. The ranges of concentrations for constructing dose-response lines were 5-50 and 50-180 mg ai l-1; and the estimated LC50 values were 12 and 90 mg ai l- for imidacloprid and lambda-cyhalothrin, respectively. The joint action of lambda-cyhalothrin and imidacloprid was studied at three ratios of 1: 1, 2: 1 and 1: 2 at three concentrations of 5, 15 and 30 mg ai l-1. The best insecticidal effect was observed at 2: 1 ratio of imidacloprid: lambda-cyhalothrin combination with 40, 69 and 90% mortality, respectively. The results revealed that lambda-cyhalothrin+imidacloprid mixture produced a synergistic effect with estimated LC50 value of 4 mg ai l-1. This may be valuable in delaying the development of resistance to either of these insecticides.

Keywords: *Myzus persicae*, synergistic effect, lambda-cyhalothrin, imidacloprid

PO247

INSECTICIDAL ACTIVITY OF 4 ESSENTIAL OILS (EUCALYPTUS) OF TUNISIAN ORIGIN ON THE EGGS OF *C. MACULATUS*

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The objective of this work is to study the toxicity of 4 essential oils (*E. cineria E. maidenii E. astringens* and *E. lehmanii*) of Tunisian origin on the eggs of *C. maculatus*. The biological parameters studied are: egg hatching rate and viability. Hatched eggs exposed to different essential oils undergo a decrease in their hatching as the dose and duration of exposure increases. The number of hatched eggs decreases as the duration of exposure increases (24, 48, 72 and 96h). After 96h, the hatching rate of the treated eggs is reduced by more than 50%. As for egg viability, after 72 hours of exposure, it varies between 37 and 62%, and this rate is less than 30% with *E. cineria* and *E. maidenii*.

Keywords: Essential oils, eggs, *C. maculatus*, Fumigation, Eucalyptus

PO248

INSECTICIDAL EFFECT AND RESIDUAL ACTIVITY OF NANOCAPSULES AND COMMERCIAL FORMULATION OF PIRIMICARB AGAINST *MYZUS PERSICAE* (SULZER)

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The inevitable use of pesticides in modern agriculture has motivated researchers to pay particular attention in developing formulations that can minimize the use of these chemicals. Nanomaterial-based formulations can reduce the use of pesticides and their impact on human health and the environment. The green peach aphid *Myzus persicae* (Sulzer) is a cosmopolitan and polyphagous pest. There are several methods for controlling aphids, but the most commonly used method worldwide is the use of insecticides. Because of frequent and extensive use of these chemical compounds, many problems have been created and management of this pest has become difficult. In this study, the aphid was reared in the greenhouse on bell pepper plants at 26±2°C, 60±5% RH, and a photoperiod of 16: 8 (L: D) h. The nanostructured lipid carriers (NLCs) nanocapsules containing pirimicarb were prepared using the hot homogenization method. The percentage of encapsulation using UV-VIS spectrophotometer was 85% which indicates the high efficiency of this method for the preparation of nanocapsules. Zeta potential studies revealed the stability of nanocapsules. The SEM images indicated that the nanocapsules were spherical in shape. The average diameter of nanocapsules was 181 nm. Bioassays were performed to compare insecticidal effect and residual activity of nanocapsules with wettable powder (WP) which is the commercial formulation of pirimicarb. The LC50 values estimated for WP and nanocapsule formulations of pirimicarb after 48 h were 216 and 73.7 mg ai/l, respectively. Comparison of the residual activity of the formulations examined revealed that WP and nanocapsule formulations of pirimicarb were active for 7 and 15 days, respectively. If similar results are obtained in commercial scale greenhouse and field experiments, it may be concluded that NLCs nanoencapsulation not only improves the efficacy of pirimicarb, it also prolongs the residual activity of this insecticide.

Keywords: *Myzus persicae*, pirimicarb, nanocapsule, insecticidal effect, residual activity

PO249

ACTIVITY OF RESIDUES OF THE CHITIN SYNTHESIS INHIBITOR TRIFLUMURON ON *HALYOMORPHA HALYS* (STÅL) NYMPHS

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Halyomorpha halys (Stål) (Rhynchota: Pentatomidae), the Brown Marmorated Stink Bug (BMSB), is a high-concern invasive species causing severe damages to orchards in many countries outside its native Asian range. Control options matching both effectiveness and sustainability are currently lacking. In this study the activity of triflumuron, an insecticide belonging to chitin synthesis inhibitors, was tested on 3rd instar nymphs of BMSB. Insects were continuously exposed to insecticide residues on potted peach plants and mortality was scored after 7, 14 and 21 days of contact. In comparison with water treated control triflumuron caused significantly higher mortality on BMSB nymphs. The percentages of mortality increased with the contact time and reached the 80% at 21 days after treatments. The effects of triflumuron on BMSB nymphal stages might be exploited for integrated management programs because of the better ecotoxicological profile of chitin synthesis inhibitors in comparison with most neurotoxic insecticides used so far against BMSB.

Keywords: Brown marmorated stink bug, Integrated pest management, Insecticides, Pentatomidae, Invasive species, Extended laboratory trials

PO250

THE APPLICATION OF SOME PESTICIDES ON *EURYGASTER INTEGRICEPS* PUT. (HEM.: SCUTELLERIDAE)

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Chemical control against overwintered adults of sunn pest *Eurygaster integriceps* Put. mostly has low efficacy. Today using insecticides and formulations that have durable toxicity on overwintered adults is more approved, because this strategy can reduce treated area against new generation. This research was conducted with six treatments and four replications in randomized complete block design against overwintered adults of sunn pest in Qazvin (Ismail Abad Agricultural Research Station) during2007-2008. Emulsions and micro capsules formulations were applied using motorized knapsack atomizer sprayer and with hands respectively. The treatments included delthametrin EC 2.5%, fenitrothion EC50%, fenitrothion G 5%, fenitrothion CS 20%, fipronil G 0.2% and check, that their effectiveness in controlling on sunn pest adults, new generation adults and nymphs were studied. Delthametrin EC 2.5% was more effective than the other treatments. Most treatments were not able to reduce damaged in the standard level (2%). Thus applying of pyrethroid insecticides such as delthametrin with micro capsulated suspension (CS) formulation can be more affected in chemical control of overwintered adults.

Keywords: Sunn pest, Formulation, Fipronil, Fenitrothion, Delthametrin

PO251

NEUROTOXICITY EFFECTS OF *MENTHA LONGIFOLIA* ESSENTIAL OIL, ACHE INHIBITION AND OCTOPAMINERGIC SITES, AGAINST TWO. INSECT MODELS, *PLODIA INTERPUNCTELLA* (LEP: PYRALIDAE) AND *TUTA ABSOLUTA* (LEP: GELECHIDAE)

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The need to control pests has involved the use of synthetic insecticides, mostly as the only effective available means. Problems associated with the use of pesticides have urged the need for biodegradable, environmentally and ecologically safe pesticides. Biopesticides based on essential oils (EOs) could be a useful alternative or complementary tool in pest control programs. In order to study the insecticidal activity of *Mentha longifolia* essential oil on *Tuta absoluta* (Meyrick) (Lepidoptera:Gelechiidae) and *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) under laboratory conditions of 27±1°C and 60±10% R.H. and a photoperiod of 16:8 h (L:D), essential oil from this plant species was extracted by Clevenger-type water distillation. The Contact toxicity of *Mentha longifolia* essential oils on fourth instar larvae of *T. absoluta* and *P. interpunctella* under laboratory conditions were investigated. Each treatment which consisted of six concentrations and a control was replicated at least eight times with 20 larvae per replicate. Probit option of SPSS was used for analyzing concentration–mortality data and estimating lethal concentrations. Mortality was recorded after 24 hours, and was corrected based on control mortality using Abbott's formula. If control mortality was more than 10%, the results were discarded and the bioassay was repeated. LC50 values of *Mentha longifolia* essential oil on fourth instar larvae of *Tuta absoluta* and *Plodia interpunctella* under laboratory conditions were 7759.55 and 127.27 ppm after 24 h, respectively. Furthermore, the possible mode of action of *Mentha longifolia* essential oil was evaluated by biochemical analysis (acetylcholinesterase inhibition) and gene expression using real-time RT-PCR analysis (the octopamine systems) in these two insect models.

Keywords: *Mentha longifolia* essential oil, insecticidal activity, AChE inhibition, octopaminergic sites

PO252

EFFECTS OF KINETIN ON HEMOCYTES OF THE MODEL INSECT *ACHROIA GRISELLA* F. (LEPIDOPTERA: PYRALIDAE)

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Kinetin is a synthetic plant growth regulator (PGR) and frequently used for the cultivating of vegetables and fruits. Plant hormones obtained from the cytokinins like kinetin could be harmful for both target and nontarget organisms in nature by the way of contact and feeding. In this respect, we investigated the effects of kinetin on hemocytes of *Achroia grisella* F. (Lepidoptera: Pyralidae) in order to better understanding of physiological impacts of PGRs on insects. Different concentrations of kinetin (5, 25, 400, and 3000 ppm) were injected on the second hind leg of last instar larvae of *A. grisella*. Controls consisted of larvae untreated, null-injected, 0.1 N NaOH-injected, PBS-injected, and injected with the mixture of 0.1 N NaOH and PBS (NaOH-PBS-injected). 24 h post-injection, obtained hemolymph from each testing larvae were applied to a Neubauer hemocytometer to detect total hemocyte counts (THC). When control groups were compared statistically only with each other it was found that THC decreased significantly in all controls except for PBS-injected according to untreated ones. Since the mixture of PBS and NaOH include the effects of other controls on THC, the results of kinetin-treated groups were compared statistically with only untreated and NaOH-PBS-injected controls. Kinetin application caused prominent decreases at all doses with respect to untreated control but the differences were only significant at 25 and 3000 ppm. Although THC were markedly affected by the injection of the mixture of PBS and NaOH, the high rate of decline at 25 and 3000 ppm doses showed that kinetin has also a perceptible role in the decline of THC. When compared kinetin-injected groups with each other, there was a significant decrease at 25 ppm according to 5 and 400 ppm. These findings demonstrate for the first time that kinetin affects the cellular immune system of *A. grisella*.

Keywords: *Achroia grisella*, kinetin, toxicity, total hemocyte count.

PO253

IMPACT OF DELTAMETHRIN TREATMENT ON LEAF MINER *LIRIOMYZA CICERINA* (DIPTERA: AGROMYZIDAE) AND THE SIDE-EFFECTS ON ITS ASSOCIATED PARASITOIDS

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Chickpea leafminer *Liriomyza cicerina* is the most dangerous pest that affecting chickpea plants. Both adults and larvae caused damage respectively through leaf punctures and laying eggs for adults and feeding into the leaf mesophyll tissue for larvae. Herein, we assessed the impact of chemical treatment using Deltamethrin (concentration 1.5ml/100 l water, Decis 50% EC) on the insect as expressed by the number of emerged adults and infestation levels. Moreover, the side-effect of this treatment was evaluated on the associated parasitoids *Opius monilicornis*, *Dilyphus isaea*, *Diaulinopsis arenaria*. The study was carried out in the experimental research station of Beja (36°44'N; 9°13'E) using a winter variety (Nour) and a spring variety (Amdoun). Trials were replicated three times through the crop season and three untreated plots served as control. Ten chickpea plants were randomly chosen from each variety, then, from each variety 50 leaves were sampled from the top, middle and the base of the plant and kept in a transparent plastic boxes under laboratory conditions until adults' emergence of both the insect pest and the parasitoids. After emergence, both insect pest and parasitoids specimens were recorded and conserved in 70% ethanol. Fly adult populations were monitored using yellow sticky traps with. The traps were placed 10 cm above the top of the plant. Results showed that Deltamethrin treatment affect both the insect pest and its parasitoids. The numbers of recorded insect were 54 and 20 adult/trap respectively for treated and non-treated plots of Nour variety; while, for Amdoun variety, insect captures reached 73 and 42 adult/trap respectively for treated and non-treated plots. The parasitoids *O. mnilicornis*, *D. isaea* and *D. arenaria* accomplished respectively parasitism rates of 8.08% 4.04% and 2.02% in treated plots of Nour variety against respectively 18.9%, 8.1% and 6.1% in treated plots of Amdoun variety.

Keywords: *Liriomyza cicerina*, Deltamethrin, *Opius monilicornis*, *Diglyphus isaea*, *Diaulinopsis arenaria*

PO254

EFFECT OF LACTONE GLYCOSIDE ON FEEDING DETERRENCE AND NUTRITIVE PHYSIOLOGY OF TOBACCO CATERPILLAR *SPODOPTERA LITURA FABRICIUS* (NOCTUIDAE: LEPIDOPTERA)

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The plant active molecules with their known mode of action are important leads to development of newer insecticides. Lactone glycoside was identified earlier as the active principle in *Cleistanthus collinus* (Roxb.) Benth. (Fam: Euphorbiaceae). It possessed feeding deterrent, insecticidal and insect growth regulatory actions at varying concentrations. Deducing its mode of action opens a possibility of its further development. A no choice leaf disc bioassay was carried out with lactone glycoside at different doses for different instars and Deterrence Indices were worked out. Using regression analysis, concentrations imparting 10, 30 and 50 per cent deterrence (DI10, DI30 & DI50) were worked out. At these doses, effect on nutritional indices like Relative Consumption and Growth Rates (RCR & RGR), Efficiencies of Conversion of Ingested and Digested food (ECI & ECD) and Approximate Digestibility (AD) were worked out. The Relative Consumption and Growth Rate of control and lactone glycoside fed larvae were compared. The concentrations needed for imparting 50 per cent deterrence was identified as 60.66, 68.47 and 71.10 ppm for third, fourth and fifth instars respectively. Presence of feeding deterrent action was confirmed by reduced Relative Consumption Rate (RCR) and Relative Growth Rate (RGR) values. Approximate digestibility (AD) was found grater indicating reduced excretion due to poor digestibility and retention of food in the gut of lactone glycoside treated larvae. Greatly reduced Efficiencies of Conversion of both Ingested and Digested (ECI and ECD) food indicated presence of toxic action. This was proved by comparing growth efficiencies of control and lactone glycoside treated larvae. Hence, presence of both feeding deterrent and toxic modes of action for Lactone glycoside was confirmed. Studies on molecular targets based on this preliminary site of action lead to new insecticide development.

Keywords: *Spodoptera litura Fabricius*, *Cleistanthus collinus* (Roxb.) Benth., Feeding deterrence, Nutritional indices, Mode of action

PO255

LARVICIDAL N-BENZYL-4-AMINOPIPERIDINE-BASED MOLECULES AGAINST *AEDES AEGYPTI* MOSQUITO WITH ANTI-CHOLINERGIC PROPERTIES

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Without available effective drug or vaccine, the viral diseases like Dengue, Zika and Chikungunya, spread by *Aedes aegypti* mosquitoes, represent a serious, medical and socioeconomic problem. Thus, their prevention is mainly achieved by vector control methods. Among them, the use of synthetic insecticides as organophosphates with the cholinergic inhibitory mechanisms has been the major tool in mosquito control. However, in recent years it has been found that some mosquito populations increase their resistance to those agrochemicals. For this reason, there is clearly a dire need for discovering new structurally different molecules with the similar action mode. Considering the above stated, in this work the mosquito larvicidal activity and acetylcholinesterase (AChE) inhibition effects of diverse twelve acyclic and cyclic N-benzyl-4-aminopiperidines against third-instar larvae of a wild strain of *Ae. aegypti* (Piedecuesta-Colombia) have been assessed. Following by WHO protocols and Ellman's procedure, we determined their larvicidal activity and could observe moderate to good activity with LC50 values of 7.9-61.1 ppm for all 12 compounds. Once the bio-efficacy of the tested molecules was confirmed, their inhibitory activity against AChE from Electrophorus electricus was examined verifying that in deed, all tested compounds could inhibit this enzyme with IC50 values of 9.0-44.0 ppm. One of them, 6-chloro-4´-methyl-3´,4´-dihydrospiro[N-benzylpiperidine-4,2´-(1´H)quinoline] with promising both larvicidal and anti-AChE activities (LC50=7.9 ppm, IC50=9.0 ppm) was also tested against cholinesterases (ChE) from *Ae. aegypti* larval homogenates. Results obtained in these *in vitro* and *ex vivo* studies clearly showed that 1) at least this compound has direct inhibitory effect on insect cholinergic system and 2) ChE inhibition was responsible for larvae death observed during the chemical treatment of larvae mosquitoes. Exhibiting the major enzymatic inhibition (78%) after 48 h exposure, this molecule is a viable structural model for the development of new larvicidal agents against *Ae. aegypti*.

Keywords: Acetylcholinesterase, *Aedes aegypti*, N-benzyl-4-aminopiperidines, mosquito larvicidal activity

Insecticide Toxicology and Non Target Effects

PO256

DIAZINON DEGRADATION BY SOIL ISOLATE BACTERIA AND MEASURING OF THE RESIDUAL CONCENTRATION IN THE PRESENCE OF THESE ISOLATES BY HPLC

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Diazinon is an organic phosphorus pesticide which has a wide range of effectiveness; therefore it has gained a lot of attention from many agricultural producers in recent years. The primary environmental concerns associated with its use namely, killing birds, surface water pollution, and destructive effects on aquatic species were taken into consideration. Biodegradation of pesticides is one of the methods for cleaning up soil, which is cost effective and adaptive with environment. Microorganisms isolated from soil samples using an enrichment culture technique have been in the minimal media where diazinon favoured as a sole carbon source. A diazinon biodegradation study was performed in liquid medium with four bacterial strains labeled S1, S2, S3 and S4 that were isolated from lately agricultural soil. All these isolates were able to entirely reduced 50 mg L-1 diazinon in mineral salt medium (MSM) as an only carbon source within 15 days of incubation. Diazinon residues were measured at continuous duration until 15 days after incubation, compared with control samples. Diazinon recovery rate was condacted at 0.1 and 1 mg kg-1, the obtained values were 80.30 and 91.80%, respectively, limit of detection (LOD) was 0.4 mg kg-1 while limit of quantification (LOQ) was 0.2 mg kg-1 Diazinon half- life values (T1/2) were 4.66, 3.76, and 3.84 and 3.87 days for S1, S2, S3 and S4, respectively and control value was 6.44 days. No significant effect on diazinon occurred with S1 (*Stenotrophomonas. maltophilia*), S2 (*Pseudomonas. stutzeri*), S3 (*Alealigen*. sp) and S4 (*Pantoea. ananatis*) treatments showed considerable effect that increased diazinon degradation rate compared with control treatment. These results highlight the potential of these bacteria to be applied in the purify of polluted pesticides wastage in the environment.

Keywords: Bacteria, Biodegradation, Diazinon, HPLC, Pesticide residue

PO257

SIDE-EFFECTS OF TWO STROBILURIN FUNGICIDES ON *NESIDIOCORIS TENUIS* AND *MACROLOPHUS PYGMAEUS* (HEMIPTERA: *MIRIDAE*)

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Making compatible the combined use of pesticides and natural enemies is one of the key aspects to achieve success in biological control programs. Currently, *Nesidiocoris tenuis* and *Macrolophus pygmaeus* are frequently used as biological control agents in various greenhouse crops. In this context, to evaluate the side-effects of pesticides used in this kind of crops on these predators is of great importance. Thus, in the present work, the strobilurin fungicides kresoxim-methyl and trifloxystrobin were tested against the adults of *N. tenuis* and *M. pygmaeus*. A negative (water only) and a positive control (chlorpyrifos) were also used. The mortality of the adult predators was evaluated by contact on fresh residue. To that end, the compounds were applied on glass substrate by using a Potter Tower. The mortality was recorded after 24, 48 and 72 h of exposure. Additionally, the sublethal effects after 72 h of exposure were evaluated, when possible (only in the case of *N. tenuis*). To do this, pairs of survival adults were used and the necessary data to obtain the parameters of the life tables were collected. The Abbott mortality after 72 h of exposure was of 100% in the case of chlorpyrifos for both predators. In the case of *N. tenuis*, kresoxim-methyl was classified slightly toxic and trifloxystrobin harmless (categories 2 and 1, according with the International Organisation for Biological Control, respectively). Nevertheless, both compounds were moderately toxics for *M. pygmaeus* (category 3). The rm values recorded for *N. tenuis* treated with kresoxim-methyl and trifloxystrobin were of 0.0874 and 0.1019, respectively. In both cases, no significant differences of this parameter were obtained with respect to the control (water only).

Keywords: *Nesidiocoris tenuis*; *Macrolophus pygmaeus*; strobilurin fungicides; kresoxim-methyl; trifloxystrobin; side-effects; intrinsic rate of increase.

PO258

Poster withdrawn.

PO259

ASSESSMENT OF INSECTICIDAL ACTIVITIES OF FARM-MADE PLANT EXTRACTS AGAINST TWO INSECT PESTS, *APHIS GOSSYPII* (HOMOPTERA: APHIDIDAE) AND *FRANKLINIELLA OCCIDENTALIS* (THYSANOPTERA: THRIPIDAE)

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We collected 13 farm-made plant extracts used for controlling insect pests from organic farms and evaluated their insecticidal activities against on 2nd or 3rd instar of cotton aphid, *Aphis gossypii* (Homoptera: Aphididae) and western flower thrips, Frankllineilla occidentalis (Thysanoptera: Thripidae) on cucumber leaf in the laboratory. Three of 13 plant extracts showed more than 70% insecticidal activities against *A. gossypii*. Insecticidal activity of ginko leaf and fruit extract against *A. gossypii* was 83.3% at the concentration of 1%. Insect flower extract showed 100, 100, 89.3 and 78.9% insecticidal activities at 1, 2, 3 and 5%, and mixed plant extract including insect flower and sweet oleander showed 96.7, 96.7, 93.3% insecticidal activities to *A. gossypii* at 1, 2 and 3%, respectively. Insecticidal activity of four plant extracts was over 70% insecticidal activities against *F. occidentalis*. Insect flower, sweet oleander, mixed insect flower and sweet oleander, and mixed garlic and wood vinegar extracts showed 73.8, 94.7, 88.0, and 74.1% insecticidal activities at the concentration of 3%. Results suggest that some farm-made plant extracts could be potential candidates for controlling *A. gossypii* and *F. occidentalis*.

Keywords: Insecticidal activity, plant extract, *Aphis gossypii*, Frankllineilla occidentalis

PO260

INSECTICIDE HORIZONTAL TRANSFER FROM TEPHRITID MALE LURES CONTAINING REDUCED RISK PESTICIDES

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Oriental fruit fly, *Bactrocera dorsalis* Hendel, is a serious pest of fruit commodities in tropical and subtropical area from Asia to Africa. Male annihilation using combination of methyl eugenol and broad-spectrum pesticides, *i.e.* organophosphate and carbamate were one of the integrated pest management tactics. Reduced-risk pesticides were introduced as killing agents in recent years due to the environmental concerns for broad-spectrum pesticides and increasing pesticide resistance of the pests. In contrast to the contact poison of broad-spectrum pesticides, many reduced-risk pesticides have strong bioactivity. Male flies fed and regurgitated insecticide-laced methyl eugenol shortly after feeding on the attractant. The objectives of this study were to evaluate four reduced-risk pesticides, chlorantraniliprole, acetamiprid, thiamethoxam and spinosad, as alternatives to organophosphate and carbamate for the fruit fly suppression and the potential of insecticide horizontal transfer from the regurgitants. The results show secondary male mortality in spinosad (75±15.6%) and thiamethoxam (70±5.8%) were significantly higher than chlorantraniliprole and acetamiprid. In addition, regurgitants collected from ME-spinosad fed males caused significantly higher female mortality (47.5±16%) than other treatments at 48 h. Cage studies were conducted to determine the attraction of reguritants collected from insecticide laced ME-fed flies. Results showed there was no statistical difference among regurgitants collected from three insecticide treatments (spinosad, thiamethoxam and acetamiprid) compared to ME only regurgitants. The results from this study demonstrated male annihilation technique with reduced-risk pesticides expands the suppression effect from individual to population level.

Keywords: Tephritidae, male annhilation, insecticide horizontal transfer

PO261

UNDERSTANDING THE GENETICS OF INSECTICIDE ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION

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The application of small molecule insecticides remains the most widespread and reliable way to control insect pests. Despite a wealth of knowledge regarding resistance mechanisms, relatively little is known about how insecticides are absorbed, distributed, metabolized, and excreted (ADME) while in route to their targets. Critical to ADME are transporter proteins from the ATP-binding Cassette (ABC) superfamily, which are capable of translocating substrates such as insecticides across cellular membranes. To this end, we genetically manipulated individual members of the ABC superfamily in the model insect *Drosophila melanogaster*, estimating their contributions to insecticide ADME through changes in insecticide toxicity level. Removal of the gene DmMdr65 gene was shown to increase susceptibility to all tested neuroactive insecticides, while deletion of the DmMdr49 and DmMdr50 genes increased or decreased toxicity depending on which insecticide was being tested. Further, overexpression or knockdown of these ABC transporters and others were achieved via the GAL4-UAS system, highlighting how expression pattern and structure influence a transporter's contribution to insecticide toxicity. These experiments represent preliminary steps to understand the genetics of insecticide ADME, which will aid rational insecticide design and resistance management.

Keywords: Drosophila, CRISPR-Cas9, ABC Transporters, ADME

PO262

PLANT IDENTIFICATION OF PESTICIDE CONTAMINATION IN HONEYBEE-COLLECTED POLLEN

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Pesticides residues are commonly found in pollen loads collected by honeybee (*Apis mellifera*) workers from agricultural landscapes. As the honeybee hive range is usually about 700 hectares, assessing the source of this contamination is not an easy task. Moreover, the residues occurring in the same pollen species may change according to the treatments during the season. Honeybees are usually loyal to a plant species while foraging, thus resulting in a plant pollen predominance in a single pollen load. Thereby, pollen loads display specific colours allowing to separate them by botanical origin. In this study, we aimed to investigate the source of pesticide contaminations from different plant species in the honeybee-collected pollen loads during apple blooming. We collected honeybee pollen loads during and after apple blooming from two beehives for each of eight localities of South Tyrol, Italy. For each hive, locality and collection period, we separated the collected pollen by the predominant colours, assessed the species predominance by palynology and performed trace analysis of the pesticide residues for each type of pollen. In

green pollen, mostly composed by apple (*Malus* sp.) (61±17%), we found residues of 8 insecticides and 17 fungicides, at least one in each sample, some of them not allowed during blossom. Orange pollen was represented by 99±0,6% and 81±17% of *Taraxacum* spp., during apple blooming and after that, respectively. *Taraxacum pollen* showed several pesticides in particularly after apple blooming, with 7 insecticides and 12 fungicides found in all the samples. Among the highest values, Chlorpyrifos and Imidacloprid reached 1.500 ppb and 150 ppb respectively, after apple blossom. As far as we know, this is the first evidence of measuring pesticide residues in botanically separated pollen loads, providing a new method of pesticide residual analysis at landscape level.

Keywords: *Apis mellifera*, palynology, pesticide residues, apple pollen

PO263

Poster withdrawn.

PO264

DNA DAMAGES IN HAEMOCYTES OF CARABID BEETLES *HARPALUS (PSEUDOPHONUS) RUFIPES* (DE GEER, 1774) – NEUTRAL COMET ASSAY IN ENVIRONMENTAL MONITORING

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Crop protection practices play a useful role to control invasive species in agricultural areas. However, concerns have been raised regarding direct or indirect sublethal effects on non-target species, even when the practices are done correctly. As a result, there is an increased need for the sensitive models to assess the impact of pesticides on organisms inhabiting agricultural areas. In this research, the neutral Comet assay was used to study the DNA damage induced by field exposure to pesticides in the haemocytes of *Harpalus (Pseudophonus) rufipes* adult from two sites. The first monitored site was a potato field located in a conventional farm (1240 m a.s.l., 39°16'58.05"N, 16°38'43.26"E, Torre Garga Farm, Calabria, Southern Italy) and treated with herbicides (Song 70® Wdg, Bismark®, Stratos®), fungicides (Curzate® 60, Cabrio® Duo, Enervin® Duo) and insecticides (Contest®) against respectively weeds, potato blight and potato beetle *Leptinotarsa decemlineata*. The control site was an organic potato field in a biological farm, located 5,5 Km north-east of the sampled conventional field (39°17'10.28"N, 16°42'28.33"E, 1150 m a.s.l.; Macchia di Tuono Farm, Calabria, Southern Italy). Analyses performed on haemocytes have shown that the levels of DNA damage recorded as tail DNA% in cell of *H. rufipes* adults from conventional treated field (71.86±2.34%) was significantly higher (Kruskal Wallis test, p □0.0001) than in beetles from the organic control site (59.74±2.62%). This result shows that *H. rufipes* is a high suitable model to monitor sublethal effects of agrochemical on non-target species. Moreover, we observed a reduction of DNA damage through the time in beetles sampled in early June compared with beetles sampled in early May in the conventional field. As a result, we assume that the high level of DNA damage recorded in May are due mainly to herbicides used before and during our sampling.

Keywords: Carabid, DNA strand-breaks, genotoxicity, ecotoxicology

PO265

LETHAL AND SUB-LETHAL EFFECTS OF LUFENURON AND ALSYSTIN ON BRINJAL SHOOT AND FRUIT BORER, *LEUCINODES ORBONALIS* (LEPIDOPTERA: PYRALIDAE)

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Brinjal shoot and fruit borer (BSFB), *Leucinodes orbonalis* is a notorious pest causes around 67% damage to brinjal crop. The larvae attack on the shoots in the early phase of the crop and later it damage the fruit. In Pakistan, farmers mainly rely on insecticides to manage this pest in the field.The assessment of lethal and sub-lethal effects of insecticides is important to interpret the overall insecticide efficacy in controlling insect pest populations. In addition to the lethal effect, sub-lethal effects may also occur in exposed insects. In this study, lethal and sub-lethal effects of two new chemistry insecticides such as alsystin and lufenuron on the development and reproduction of brinjal shoot and fruit borer were evaluated in the laboratory. The results indicated that lethal and sublethal concentrations of both insecticide increased the duration of larval and pupal stages as compared with the control treatment. The percent pupation and percent adult emergence were significantly lower in insects treated with lethal and sub-lethal concentrations of both insecticides as compared with control. The lethal and sub-lethal concentrations of lufenuron and alsystin decreased the pupal weight of the treated insects as compared with control. The proportion of pairs that produced eggs was not significantly different between treatments. The lethal and sub-lethal concentrations of lufenuron and alsystin also decreased the fecundity of the treated insects as compared with control. The results from this study will be helpful to develop the strategy to incorporate these two insecticides in an integrated pest management programme.

Keywords: Lethal, Sub-lethal, Brinjal fruit and shoot borer, Lufenuron, Alsystin

PO266

EFFECT OF THIAMETHOXAM ON TOTAL HEMOCYTE COUNTS OF *GALLERIA MELLONELLA*

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The aim of the present study was to investigate the effects of thiamethoxam on hemocytes content in the model organism *G. mellonella* larvae. Larvae were reared under laboratory conditions at a temperature of 28°C±2°C and a relative humidity of 70%±5% in a dark cycle. Thiamethoxam (10, 20, 30, 40, and 50 µL) was injected into the last instar larvae and total hemocyte count was calculated by Neubauer hemocytometer at 24, 48, 72, and 96 hours following injection. Hemocytes counts decreased significantly after the injection of thiamethoxam except from dose of 10 µL at 24, 48, and 72 h. However, at 96h, all doses of thiamethoxam caused a significant decrease in total hemocyte count when compared with the control. This result shows that thiamethoxam has a destructive effect on the hemocytes which are immune system component of the model organism, *G. mellonella*. The results of this study provide indirect information about the possible immunotoxic effects of thiamethoxam in nontarget organisms.

Keywords: Thiamethoxam, total hemocyte count, *Galleria mellonella*

PO267

FUMIGANT TOXICITY OF ESSENTIAL OIL FROM *ARTEMISIA SIEBERI*, *EUCALYPTUS CAMALDULENSIS* AND *THYMUS KOTSCHYNUS* AGAINST GERMAN COCKROACH, *BLATTELLA GERMANICA* (ORTHOPTERA: BLATTELLIDAE)

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The intense application of chemical insecticides has led to the development of insecticide resistance in population of *Blattella germanica* L. (Orthoptera: Blattellidae), a cosmopolitan household pest. Therefore, it is important to search for new active compounds as alternative to conventional insecticides. The objective of the current study was to determine the fumigant toxicity of essential oils, *Artemisia sieberi* Besser, *Eucalyptus camaldulensis* Dehn. and *Thymus kotschynus* Boiss. & Hohen against adult stage of B. germanica at 1.67 to 66.67 µL/L air. Dry ground leaves were subjected to hydrodistillation using a modified Clevenger-type system. The experiment was conducted in a standard environmental condition set at 25±2ºC, 70±5% RH and photoperiod of 16:8 h, L:D. The mortality was recorded 24 h after treatment. Data probit analysis demonstrated that the lethal concentration to cause 50% mortality (LC50) and their 95% confidence limits of *A. sieberi*, *E. camaldulensis* and *T. kotschynus* were 17.30 (12.73-21.80), 21.83 (17.43-26.53) and 28.80 (22.83-34.47) µL/L air, respectively. According to the results, the LC50 value were significantly higher in *A. sieberi* and *E. camaldulensis* than that of *T. kotschynus*. The findings indicate the potential role of *A. sieberi* and *E. camaldulensis* as fumigant insecticides against *B. germanica*.

Keywords: Mortality, Botanical pesticide, LC50, German cockroach, Fumigation

PO268

SEASONAL OCCURRENCE OF PEST AND EFFICACY OF INSECTICIDE CONTROL IN SCHISANDRA BERRY (*SCHIZANDRA CHINENSIS* BAILLON) IN SOUTH OF KOREA

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Schizandra chinensis Baillon is a deciduous shrub vine belonging to the genus Schisandraceae. It is known to contain 49 species of 2 genus(Schisandra, kadsura) in the world, and is distributed mostly in tropical Asia and the Malay archipelago. In Korea, there are 3 species of 2 genus. *S. chinensis* harvests fruits in September and mixes with sugar to eats juice or it is dried then used as medicine. Pests of 1 species of Diptera, 1 species of Homoptera, 6 species of Lepidoptera, 2 species of Hemiptera and 2 species of Thysanoptera had occurred at ochards of *S. chinensis* and it cause damage to stems, leaves and fruits. The Major damaging pests are *Scirtothrips dorsalis* Hood, which occur in May to September. As a result of the insecticide test, 5 insecticide were effective, emamectinbenzoate EC was effective in ochards of *S. chinensis*.

Keywords: *Schizandra chinensis* Baillon, *Scirtothrips dorsalis* Hood, Insecticide Control

PO269

MODELING SPIDER MITE RESURGENCE AT POPULATIONS LEVEL

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Mortality and median lethal dose/concentration (DL50/CL50) are used as toxicological endpoints in 95% of published studies in the SELCTV database. The end result is an oversimplification of the potential consequences of pesticide-induced stress. Nowadays there is a general consensus that the demographic approach provides better toxicological information than lethal dose estimates. This method has been now adopted in many laboratory studies since the approval of the EU directive 2009/128/EC on the sustainable use of pesticides. The novelty we would like to introduce is how to incorporate a demographic approach in semi-field and field studies for pesticide-effect assessment. Here we present a case study where matrix population models are used for evaluating two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) resurgence after insecticide application. The obtained data, consisting of population time series, were used to generate a stage-classified projection matrix. Most of the methods utilized for estimate the parameter values of stage-classified models rely on following cohorts of identified individuals. However in this study the observed data consisted of a time-series of population vectors where individuals are not distinguished. The relationship between the observed data and the values of the matrix parameters that produced the series involves an estimation process called inverse problem. The set of parameters that minimize the residual between the collected data and the model output for the study presented here was estimated using the quadratic programming method. The set of estimated parameters support the hypothesis that some insecticides, namely etofenprox, deltamethrin and beta-cyfluthrin, fostered a higher mite population growth than the untreated control. This was principally due to higher adult fecundity and egg fertility, clearly explained by Life Table Response Experiments, performed starting from estimated matrices, indicating a likely trophobiotic effect.

Keywords: Matrix population model, Spider mite resurgence, Life Table Response Experiments, *Tetranychus urticae*, Integrated Pest Management, Side-effects.

PO270

SUBLETHAL EFFECTS OF INSECTICIDES ON *TRICHOPRIA DROSOPHILAE*, A PUPAL PARASITOID OF THE INVASIVE SPOTTED WING DROSOPHILA

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Integrated Pest Management (IPM) of invasive insect pests mainly relies on the combined effects of agrochemicals and on the ecological services offered by natural enemies. We assessed the non-target effects of low-lethal insecticide concentrations toward the generalist pupal parasitoid of drosophilids, *Trichopria drosophilae* (Hymenoptera: Diapriidae). The tested insecticides belong to six chemical classes, and were applied at their label and Lethal Concentrations 10 (LC10), previously estimated for larvae and adults, of the invasive pest, *Drosophila suzukii* (Diptera: Drosophilidae). When insecticides were applied at field concentrations in insecticide-contaminated diet, no or very few *D. suzukii* developed. Whereas, parasitoids readily attacked host puparia contaminated with LC10 of insecticides, but suffered great sublethal effects. The parasitoid development, size, fertility and progeny sex-ratio were strongly affected by most of the tested chemicals. The survival and the fertility of parasitoids exposed to dry residues of insecticides, both at label and LC10 estimated for *D. suzukii* adults, was affected only by the three organophosphate insecticides. The results provide evidences of multi-trophic cascade effects of some insecticides that are proposed in IPM schemes.

Keywords: Biocontrol, *Drosophila suzukii*, ecotoxicology, risk assessment

PO271

DO PARASITES MODULATE THE OUTCOME OF NEONICOTINOID LD50 TESTS IN BUMBLEBEES?

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Neonicotinoids are the most widely used insecticide around the globe. However, an increasing number of studies have documented that they have negative impacts on important managed and wild pollinators. Bumblebees, as one of the most important groups of pollinators, have been the focus of significant research, with neonicotinoids being shown to impact learning, foraging, colony foundation, colony growth, and colony reproduction. The vast majority of these studies have examined the impacts of neonicotinoids in isolation, but in the real world their effects may be modulated by interactions with other stressors. Parasites, which are ubiquitous in natural populations, represent one such stressor. Here we use the lethal dose experimental protocol, which underpins current policy decisions on the use of agrochemicals, to investigate how natural parasites of bumblebees may modulate the impacts of neonicotinoids on bee health. Lethal dose experiments are a standardized method of testing at what concentration the chemical in question becomes lethal. Using the common and abundant bumblebee, *Bombus terrestris*, and naturally occurring parasites, including the trypanosome *Crithidia bombi*, we conducted a fully-crossed LD50 experiment, with the neonicotinoid thiamethoxam acting as the chemical under investigation. We recorded the lethal dose under both the absence and presence of the parasite. We also recorded sucrose consumption, for the duration of the LD50, to determine whether changes in the dose rate of the neonicotinoid had any effect on the workers feed intake. Finally, we measured parasite intensity after the LD50 to determine whether the presence of the neonicotinoid at different dose rates has any effect on the intensity of the parasite. We discuss our results in the context of pesticide regulation.

Keywords: Bumblebee, Neonicotinoid, Pesticide, Parasite, LD50, *Crithidia bombi*, Insecticide

PO272

STUDY ON THE EFFECTS OF WATER QUALITY ON CHLORPYRIPHUS (EC 40.8%) AND IMIDACLOPRID (SC 35%) QUALITY CONTROL INDEX

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Water is used as an important carrier for the pesticide spraying. Physical and chemical properties of water can affect the quality control indexes and the efficiency of pesticides. So, the study of the water characteristics of different regions of the country is very necessary. For this purpose, water samples from Mazandaran, Fars, Isfahan, Markazi, Gilan, Qazvin, Kerman (two regions), Khorasan Razavi, Yazd and Tehran provinces were collected. The effects of water hardness, pH and electrical conductivity (ec) on the quality control of two types of insecticides such as chlorpyrifos (EC 40.8%) and imidacloprid (SC 35%) as common and high-consumption formulation of the country were stuided. The results of water hardness test showed that water sample from Kerman with 2255 ppm and then water of Shiraz with 744 ppm had the highest hardness and sample of water in Guilan region with 60 ppm had the lowest degree of hardness in water tested areas. The emulsion stability of chlorpyrifos in water samples from Qazvin, Gilan and Khorasan Razavi (with low water hardness), showed no creaming, while in areas with high hardness such as Kerman, Shiraz and Yazd, Creaming was high in the solution. The suspensibility of imidacloprid in water samples showed that suspension in Kerman, Shiraz and Yazd water samples was unacceptable and the others areas was acceptable. The above study shows the high effect of water quality on the quality control indexes of insecticides, so it is necessary to increase the studies of this kind of investigation.

Keywords: Water hardness, water pH, quality control, insecticides

PO273

TOXICITY AND SUB-LETHAL EFFECTS OF SOME PESTICIDES ON *ENCARSIA FORMOSA*

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The aphelinid wasp, *Encarsia formosa* Gahan (Hym: Aphelinidae) has been successfully used, either singly or in combination with other control methods, for management of whiteflies around the world. In this study, the toxicity of two commonly used pesticides (buprofezin SC 40% and Abamectin EC 1.8%) and a plant-derived extract, taken form aerial parts of *Echinophora platyloba* (Umbelliferae), to different life stages (larval, pupal, and adult) of *E. formosa* was investigated under laboratory condition. Using laboratory bioassays, the median lethal doses (LD50) of abamectin, buprofezin and *E. platyloba* extract were determined as 724.20, 280.90 and 651.8 mg/L for larval stage, as 290, 820.75, and 901.1 mg/L for pupal stage, and as 306.27, 3309.15, and 831.7 for adult stage of the parasitoid, respectively. The effects of sub-lethal dose (LD30) of the insecticides on some biological parameters of the parasitoid were investigated when the wasps were exposed to the insecticides at different life stages. Pre-adult development time was negatively affected by all insecticides ($P<0.01$), with more intense effects were recorded for buprofezin and abamectin. Treatment of larvae and pupae with buprofezin and abamectin resulted in significant decrease in adult longevity ($P<0.01$); however the effect of *E. platyloba* on longevity was significant only following pupal treatment. Adult fecundity was not affected by larval and pupal treatment with any of insecticides ($P>0.05$). However, the wasps experienced decreased fecundity when exposed to abamectin, and to a lesser extent *E. platyloba* extract, during adulthood ($P<0.01$). Results of this study indicate that sub-lethal doses of *E. platyloba* extract and buprofezin have less harmful effects on life parameters of this biological control agent. Given the specificity of buprofezin for immature stages of insects and with respect to safety of botanical pesticides for non-target organisms, these compounds can be used in integrated management of whiteflies in greenhouses.

Keywords: Sub-lethal effects, greenhouse whitefly, bioassay, herbal insecticide, IPM

PO274

THE CONSUMPTION OF *EUPATORIUM BUNIIFOLIUM* ESSENTIAL OIL DOES NOT AFFECT SURVIVAL OR ETHYL OLEATE PRODUCTION IN HONEYBEES

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In the search for alternative control agents for *Varroa destructor*, the ectoparasite that may contribute to the honeybee colony collapse disorder we have selected the essential oil (EO) from *Eupatorium buniifolium*. The vapors from this EO showed fumigant activity against varroa with no toxicity for bees in laboratory assays as well as moderate activity in a preliminary field trial. These results prompted us to further investigate on other potential acute toxic and sublethal effects of this EO on bees when applied by different means. The acute toxic activity (LD50) was estimated by: a) a "Complete Exposure Test" and b) a topic toxicity test (EPA, OCSPP 850.3020, 2012). In the first case, the LD50 was 0.15 mg/cm^2 (fiducial interval 0.11-0.18, p <0.01); and the LD50 according to the EPA protocol was 252 µg/bee (fiducial interval 207-318, p <0.01) indicating that this product can be considered safe (EPA recommendation for safe products is a LD50>25 µg/bee). The chronic effects by ingestion was also studied following the The Coloss Beebook guidelines. In this assay, the EO was supplied in the diet of 2-day old bees for 12 days. During the assay, the food consumption was not different among bees fed with the EO at different doses (300-6000 ppm) and the control bees (ANOVA, p <0.01); neither was the survival among groups (Kaplan-Mayer analysis). Besides, at the end of the assay the production of ethyl oleate, a primer pheromone that regulates the worker behavioral changes and that has been reported to change under stress conditions, was quantified (GCMS). Our results showed that bees that had consumed the EO exhibited similar titers of ethyl oleate as control bees (ANOVA, GLM, P >0.05). These results point to the *E. buniifolium* EO oil as a good candidate to develop a botanical acaricide.

Keywords: *Apis mellifera*, Asteraceae, Varroa, pheromones

PO275

NOVALUROM INSECTICIDE CAUSES TOXIC EFFECTS IN SILKWORM *BOMBYX MORI* (LEPIDOPTERA: BOMBYCIDAE)

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The silkworm, *Bombyx mori* is the most important insect for silk production. It feeds on mulberry leaves and is highly sensitive to agrochemicals, thus the use of insecticides in crops surrounding the mulberry plantations can affect the creation of *B. mori*, leading to reduction of egg fertility, cocoon quality and causing his death. Novalurom is an insecticide that inhibits the synthesis of chitin in insects and used in the control of insect pests of crops near mulberry plantations. We investigated the effects of Novalurom in the development of *B. mori*. Larvae were selected into two experimental groups: control group (CG) and treatment group (TG: treated with sublethal concentration of 0, 15 mL/L Novalurom). After ecdysis from the 2nd to the 3rd instar, the TG larvae were fed for 24 hours with mulberry leaves treated with the insecticide. In parallel, a new exposition was carried out, however in larvae that carry out the ecdysis from the 4th to the 5th instar. After exposure, we analyze symptoms manifested; mortality rate; weight of the cocoons (full and empty/pupae); percentage of defective cocoons; number of eggs oviposited. We observed rupture in the integument, complete cessation of feeding, late development, incomplete ecdysis and presence of rosary-shaped excrements. After 240h of exposure, there was a 100% mortality in TG larvae from 1st exposure and 20% mortality from 2nd exposure. There was reduction in the weight of the cocoons constructed by TG, as well as construction of defective cocoons (53.8% in males and 64.3% in females); the CG did not produce defective cocoons. We also observed that the number of eggs oviposited by females of the TG were significantly lower than the GC. We conclude that Novalurom affects the development of *B. mori* and production of the silk cocoons, which can cause serious damage to the sericulture.

Keywords: Silkworm, novalurom, toxicity, mortality.

PO276

AUTOMATED ANALYSIS AND SORTING OF INSECTS BY LARGE PARTICLE FLOW CYTOMETRY

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Toxicological screening of insecticides and genetic studies on insects and parasites require the need for automation and higher throughput technologies. Large particle flow cytometers from Union Biometrica provide automation for the analysis and dispensing of insects (*e.g.* mosquito and drosophila), small model organisms (*e.g.* C. elegans), parasites, seeds, and fragile cells and cell clusters that are too large or fragile for traditional flow cytometers. The organisms in the sample are analyzed one by one while they travel through the flow cell. The size (TOF), optical density (EXT), and the intensity of fluorescent markers, if present, is captured. Once analyzed, objects can be sorted according to user-selectable criteria, and dispensed into stationary bulk receptacles or multi-well plates for high-throughput screening. COPASTM and BioSorter® instruments have been proven to analyze and sort large objects with a higher speed and precision than present manual techniques. By automating the currently, time consuming manual processes, the time required for experiments is dramatically reduced, human error is eliminated, and new experiments that previously could not be considered are now possible. Here we demonstrate the possibilities of this technology using drosophila and mosquito larvae.

Keywords: Flow cytometry, screening, toxicology, automation

PO277

LONG CHAIN FATTY ACID COENZYME A LIGASE (FACL) REGULATES TRIAZOPHOS-INDUCED STIMULATION OF REPRODUCTION IN THE SMALL BROWN PLANTHOPPER (SBPH), *LAODELPHAX STRIATELLUS* FALLEN

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The small brown planthopper (SBPH), *Laodelphax striatellus* Fallen is a major pest insect of rice, wheat, and maize in China and other countries. SBPH not only damage rice plants through sucking plant sap, but also transmits rice virus diseases, for example, striped virus disease (RSV), black streaked dwarf, and maize rough disease virus. Therefore, understanding of pesticide-induced stimulation of reproduction in SBPH is of great significance for the pest management. Our previous study discovered that triazophos (TZP) increased reproduction of SBPH. But the molecular mechanisms are unclear. Here, by using proteomic analysis, we screened and cloned the gene of long chain fatty acid coenzyme A ligase (FACL), and silenced FACL to examine influences of TZP on reproduction and glycerin content in SBPH females. In TZP-treated females *vs* control females, there were 41 differential proteins in 18 pathways related to reproduction, of which 8 were up-regulated and 33 were down-regulated. TZP+dsFACL eliminated TZP-induced stimulation of reproduction of SBPH females (↓about 73.92%) and decreased glycerin content and body weight (↓about 19.93% and 13.62%). TZP+dsFACL treatment led to reduced expression of FACL (↓about 61.88%). FACL is a key gene of TZP-induced increase of reproduction of SBPH.

Keywords: Long chain fatty acid coenzyme A ligase, the small brown planthopper, reproduction, striped virus disease, triazophos

PO278

THE PRACTICAL METHODS FOR REDUCTION OF INSECTICIDE RESIDUES ON VEGETABLES AND FRUITS

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Globally widespread application of insecticides has caused residues on freshly foods. They have potential toxic effects on non-target organisms including human. Although several novel insecticides, which are environmental friendly and safe for non-target organisms, have been began to use in developed and developing countries, broad spectrum synthetic insecticides, *i.e.* organophosphorus, carbamates, pyrethroids, neonicotonids, are still the most commonly used compounds for the control of the key pests of many vegetables and fruits. A number of chlorinated hydrocarbons, organophosphorus and carbametes insecticides have been restricted in EU countries, USA and Turkey, the use of these compounds have still been allowed in a large part of the world. Depending on the combined effects of some factors such as pH, temperature, microbial, metabolic and photo-degradations, insecticides can be disrupted within variable durations. Nevertheless, some residues of the compounds would be found on fresh crops. Various food processing and home preparation activities have some effects on removing these residues. These techniques are cooking, electrolyzed oxidizing, electrolyzed reducing, hydrostatic pressure, juicing, ozonation, peeling, sonication, storage, washing with several non-toxic solutions and combined techniques (washing & peeling & canning, washing & peeling, washing & cooking, washing & boiling, ozonation & ultraviolet & oxidation, washing & blanching & cooking). The current reviewing demonstrated that the most degradation potential (with 60-90% reduction) was found at frying, electrolyzed oxidizing electrolyzed reducing, ozonation peeling, sonication, acetic acid and salt solutions or a combined technique (ozonation & ultraviolet & oxidation). But the degradation potentials of these methods were effected with chemical features of each insecticides. Based on several scientific studies, these differences among insecticides belong to different chemical groups, *e.g.* Carbamates, Neonicotionids, Organochlorines, Organophosphorus, Synthetic Pyrethroids, Phenylpyrazole, were given at this presentation. The degradation potentials and rates of these techniques were compared with each other.

Keywords: Insecticides, degradation, residue, vegetable, fruit, reduction

Session 6.
Ecology, Biodiversity and Conservation

Arthropod-Mediated Ecosystem Services in Agriculture

PO279

ASSESSING THE IMPACT OF AGRICULTURAL STRATEGIES ON SOIL ARTROPODS: A CASE STUDY USING ENVIRONMENTAL DNA

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Elucidating how agricultural practices affect soil arthropod's communities is of relevant for both scientific and economic interests. Thus, using DNA metabarcoding approach, Arthropods communities inhabiting soil of organic and conventional farms were characterized. Soil samples were collected from organic and conventional farms, covering the margin and three levels towards the center of a stable meadow and a barley field in both farms; each sample consisted of 10 homogenized cores of soil (~560 cm^3), sampling was performed in spring, summer and autumn Soil texture, pH, N and C parameters were measured. DNA was extracted from three replicates of each soil sample. A fragment mitochondrial cox1 was amplified using three primer pairs and sequenced using Illumina Miseq. Raw sequences were processed and analyzed using Qiime to obtain Operational Taxonomic Units (OTUs) table. Approximately 75% of the obtained reads were identified as Animalia, and among these ~80% as Arthropods. α-diversity indices barley field in conventional farming were significantly lower (OTUs= 854, H'= 5.34±0.53, Pielou's evenness= 0,77±0,06) comparing with the other samples (OTUs>1100, H'>6 and Pielou's evenness>0,8). Fitting the farming system, the field, position as factors in NMDS showed that Arthropods communities were not affected by the collecting season and the farming system (organic *vs* conventional), instead crop *vs* stable meadows and the position in the field (margin *vs* middle) have a strong effect. Soil properties affected the Arthropods communities, especially the pH on Chilopoda and Diplopoda and the C/N ratio on Arachnida and Insecta. Our results pointing out that the strategy of farm management does not affect the arthropod communities of the soil as much as the soil properties itself, while the position in the filed had a major effect, highlighting the importance of green corridors for maintaining the soil biodiversity and the agroecosystem functioning.

Keywords: Soil Arthropods, DNA metabarcoding, agroecosystems, biodiversity

PO280

BUMBLEBEES DO NOT EXPAND THEIR DIET UNDER REDUCED WORKFORCE, AS REVEALED BY POLLEN DNA BARCODING

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Pollinators are affected by a high amount of stressors, as parasites, diseases, lack of food resources and some agricultural practices, including pesticides. Among pollinators, bees are particularly dependent on the flower resources, since they collect pollen and nectar for feeding the larvae. However, during their life cycle, social bees might undergo workforce losses because workers could be overpowered by environmental stressors. It might be expected that the remaining workers compensate the lack of incoming resources by individually collecting more resources per foraging trip. In this study, commercial colonies of the bumblebee *Bombus terrestris* were experimentally manipulated by removing half of the workers, in order to investigate changes in foraging strategies. Before and after the manipulation, the pollen pellets from the curbiculae were collected from the individual workers returning to the nests after their foraging trips in a flower-rich natural area in the Czech Republic. The manipulated colonies were compared with untreated ones in the same period. Meta-barcoding of the pollen's DNA was performed by targeting the ITS2 region using High throughput Sequencing (HTS). From the plant species associated to each bumblebee worker, the ecological network was derived and changes in niche breadth and network structures were tested. Overall, bumblebees were feeding on 34 plant taxa, revealed by DNA barcoding. However, only minor changes in the diet breadth of the bumblebees or in the feeding networks were found after the manipulations. At the end of the experiment, the manipulated colonies were smaller and without new queens. These results may suggest that bumblebees lack of plasticity in individual foraging, because they do not expand their foraging niche when less resources arrive in the nest due to workforce losses, with implications for population size.

Keywords: Bumblebees; *Hymenoptera Apidae*; Network Analysis; Foraging; DNA barcoding of diet; Feeding; Beneficial insects; Social insects; Pollinators

PO281

VALORISATION OF CHICKEN MANURE USING INSECTS: *HERMETIA ILLUCENS* IN THE VALORIBIO PROJECT

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Over the last decades, the need to manage organic waste in a more efficient way and the need to find new sources of energy have opened new horizons in the use of insects for various purposes (*e.g.* food, feed, biodiesel). The ValoriBio project focuses on the valorisation of chicken manure through the use of *Hermetia illucens* (Diptera, Stratiomyidae), for the production of high quality compost and bioplastics for agricultural purposes. This study is aimed at the optimisation of the growth parameters of *H. illucens* on a substrate formed by a mixture of chicken manure, zeolitic tuff (Ca-chabazite), soil improver obtained from pruning shears of urban green, and water. The addition of the Ca-chabazite aims to reduce unpleasant smells, trap the excess of ammonium, and contribute to the formation of a post-breeding substrate which can be used as high-quality compost. The parameters to be maximized were: percentage and maximum average weight of prepupae and percentage of emerged adult flies. Results of a first trial, based on a special cubic model of combined mixture design that tested different ranges of the substrates at 27 and 33°C, recommended the removal of the soil improver and the selection of 27°C as preferred rearing temperature. A second trial considered different ranges of chicken manure, Ca-chabazite (at two different particle sizes) and water. Results from this experiment allowed the definition of the optimal composition for the substrates to obtain the highest percentage of prepupae (71-74%) and the highest average prepupae weight (0.069-0.072g), and were therefore used to plan the validation test, where chicken manure ranged between 34.5 and 45.0%, Ca-chabazite (larger particle size) between 5.0 and 7.2%, and water between 50.0 and 58.3%. These results are the basis to develop an optimized rearing cycle of *H. illucens* in an automatized pilot plant for organic waste conversion.

Keywords: Black soldier fly, organic waste mass reduction, soil improvers, zeolitic tuff, bioplastic mulching sheets, circular economy

PO282

ORGANIC *versus* CONVENTIONAL MANAGEMENT IN POTATO FIELD: EFFECTS ON CARABID BEETLES COMMUNITY

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Carabid beetles are well known to be an important group of beneficial arthropods in agroecosystems involved in pest control. In this study, we monitored a conventional and an organic farm, located in the agricultural landscape of Sila Mountain, in order to quantify the carabid species assemblage in potato fields and to compare effects of different agricultural management. The conventional potato field (39°16'58.05"N, 16°38'43.26"E, 1240 m a.s.l. Torre Garga, Calabria, Italy), was treated with herbicide (cycloxydin) before potatoes were planted, with pesticides (imidacloprid and alpha-cypermethrin) and fungicides (cymoxanil, pyraclostrobin and dimethomorph) during the growth phase of potatoes. The organic potato field (39°17'10.28"N, 16°42'28.33"E, 1150 m a.s.l. Macchia di Tuono, Calabria, Italy) was sprayed with pesticides (pyrethrin) and fungicides (copper oxide) during the potato growth phase. In spring and autumn 2017 carabids have collected using 12 pitfall traps for each field (20 species, 765 individuals). In the conventional potato field, 11 species in 268 individuals were identified, with 5 dominant species: *Harpalus (Pseudoophonus) rufipes* (De Geer, 1774), *Calathus fuscipes* (Goeze, 1777), *Amara apricaria* (Paykull, 1790), *H. distinguendus* (Duftschmid, 1812), and *H. decipiens* (Dejean, 1829). In the organic field, 17 species in 497 individuals have been recognized, with 7 dominant species: *H. serripes* (Quensel in Schönherr, 1806), *H. decipiens*, *H. distinguendus*, *C. fuscipes*, *H. sulphuripes sulphuripes* (Germar, 1824), *H. rufipes*, and *Calathus cinctus* (Motschulsky, 1850). Our results suggest that the use of herbicides and insecticides in the conventional potato field have an indirect effect mainly on activity of carabid species that are natural predators of invasive pests (weeds and arthropods). Thus, the species structure and abundance of carabid species in potato fields might be related to the agricultural practices.

Keywords: Carabid beetles, pesticides, abundance, activity, potato field

PO283

DIET BREADTH MODULATES PREFERENCE - PERFORMANCE RELATIONSHIPS IN A PHYTOPHAGOUS INSECT COMMUNITY

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In most phytophagous insects, larvae are less mobile than adults and the success of their development depends on the quality of the plant chosen by the adult. According to the "mother knows best hypothesis", adult preferences are expected to be correlated to performances of larvae, maximizing fitness with adults choosing plants were larvae have optimal development. However, a correlation between adult preference and larval performance is not always found in empirical studies and a meta-analysis suggested that this correlation differed with the level of specialisation, *i.e.* specialists have a stronger preference for high quality host plant species compared to generalists (Gripenberg *et al.* 2010). The aim of our study was to test empirically whether the relationship between female preference and larval performance was identical for specialist and generalist species from a same community. We studied six species of fruit flies (Diptera: Tephritidae) occurring in La Réunion (France) including four generalist and two specialist species. We measured in laboratory the female fecundity (preference) and the larval survival (performance) on 29 host plants present in La Réunion belonging to 15 families. We, then, evaluated the relationship between the female preference and the larval performance. As expected, preference-performance relationship differed according to the degree of specialisation with a strong positive correlation for specialists and no relationship for generalists. Our study brings empirical evidence that the preference-performance relationship differed according to the degree of specialization. Host selection is useful to specialists that are more adapted to survive on their host plants compared to generalists. However, choosing the best plant on which to oviposit has a cost in term of time and energy especially for generalists that are facing many variable stimuli from different plants which may explain their non-selectivity. Generalist females may have no benefit to undergo costs that implies the selection of hosts.

Keywords: Host range, Host selection, Larval survival, Oviposition choice, Specialisation

PO284

INFLUENCE OF COVER CROPS TERMINATION ON THE ACTIVITY OF BENEFICIAL GROUPS OF GROUND DWELLING ARTHROPODS

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Cover crops in rotation and related termination techniques, can affect the arthropod dynamics of the cash crops, including both bottom-up (*i.e.* insect pests) and top-down mechanisms (*i.e.* ecosystem functions like biological control). The aim of this study is to monitor the soil biodiversity in order to point-out the composition and spatio-temporal dynamics of arthropod fauna, as consequence of the use of cover crop in rotation, including its termination. Two experiments were carried out in Italy, to compare the influence of a cover crop introduced before cash crops (cauliflower and tomato), and terminated by roller crimper (flattening) or by green manure, on soil arthropods abundance and pest-beneficial insect diversity. Results demonstrate that termination techniques strongly affected the activity density of important taxa, leading to a better conservation of soil predators. Carabidae (Coleoptera) benefited from roller crimper termination on cauliflower and tomato in one of the two year of trials and this trend was driven by the generalist species Pseudophonus rufipes. Roller crimper termination also enhanced rove beetles activity density on cauliflower (both seasons) and tomato (first year). No differences were detected on cauliflower for ground dwelling spiders, but this group resulted more abundant in green manure on tomato on both year of trials. Roller crimper technique can be a valuable alternative to green manure to terminate a cover crop in no-till management, improving the activity density of important predator groups (Carabidae, Staphylinidae).

Keywords: Cover crops, Carabidae, Staphylinidae, ground dwelling arthropods

PO285

POPULATION FLUCTUATIONS OF ERIOPHYID MITES AND THEIR PREDATORS ON TWO VARIEITIES OF OLIVE, TURKEY

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Olive is an agricultural product which can be processed as olive oil and table olive and grown commonly in Mediterranean countries. There are many factors; pests, diseases etc. that limit the production of olives. Eriophyid mites have been found to cause significant damage in olive production areas in recent years. Therefore, this study was conducted to determine population fluctuations of eriophyid and their predators in two olive orchards including ayvalık and memecik cultivars in Izmir (Kemalpasa), Turkey in 2016-2017. Orchards were regularly visited every week between March and December and leaves, buds and fruits were collected from the trees. As a result, eriophyid mites, *Aceria oleae* and *Tegolophus hassani* (Eriophyidae) were found widely in the spring (April-May) and autumn (September-October) in both cultivars. The population of eriophyid mites were obtained more in ayvalık variety than in memecik variety. A total of five species were detected as predators: *Typhlodromus (Anthoseius) athenas*, *Typhlodromus (Typhlodromus) psyllakisi*, *Typhlodromus (Typhlodromus) athiasae* (Phytoseiidae) and *Agistemus duzgunesae* (Stigmaeidae), *Raphignathus gracilis* (Raphignathidae). The most common predatory mite was *Thyphlodromus athenas*. Further studies on the efficacy of these predatory mites on the eriophyid mites should be carried out.

Keywords: Olive, Eriophyidae, Phytoseiidae, Stigmaeidae, Ayvalık, Memecik, Turkey

PO286

REGIONAL SCALE SURVEY ON CICADOMORPHA AND FULGOROMORPHA PRESENT IN APPLE ORCHARDS IN SOUTH TYROL (ITALY)

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The quarantine pest Apple Proliferation (AP) is one of the economically most important diseases in European apple cultivation. It is caused by the cell-wall less bacteria ‘*Candidatus Phytoplasma mali*’, which is spread by the phloem-sucking psyllids *Cacopsylla picta* and *C. melanoneura*. In South Tyrol (Northern Italy), severe outbreaks were documented since the 1990s that led to high economic losses in the region. In some years the infestation rate of AP does not correlate with the presence of the known vectors, implying the presence of other, so far unknown transmitting insects. Vectors of phytoplasma diseases until now are exclusively found in the order of Hemiptera. Thus, elucidating the species community of Fulgoromorpha and Cicadomorpha at a regional level is of great interest, to lay the groundwork for further investigations on identifying vectors possibly involved in AP disease spread. In a three-year monitoring period, 52 apple orchards located in main area of AP occurrence in South Tyrol were surveilled. More than 31,000 specimens were collected applying the beating tray to collect the specimen from tree canopy, as well as the insect net to capture species related to the herb layer in the understory vegetation and nearby surroundings. A total of 95 Auchenorrhyncha species was recorded in the survey. Highest abundances were recorded for *Laodelphax striatella*, *Empoasca vitis*, *Edwardsiana rosae*, *Psammotettix alienus*, *Macrosteles sexnotatus* and *Dicranotropis hamata*. Several invasive species, such as *Metcalfa pruinosa* and *Orientus ishidae* were sampled in the investigated area. Some species captured during the field study are known to be vectors, or potential vectors of phytoplasma diseases, such as *Fieberiella florii*, *Anaceratagallia ribauti* and *Reptalus panzeri*. The faunistical survey provides the basis for the targeted selection of “interesting” species, which will be analyzed by qPCR to assess their natural infection rate with the causative agent of AP.

Keywords: Auchenorrhyncha, biodiversity, phytoplasma, insect vectors, Apple Proliferation

PO287

COMPARING THE EFFECTIVENESS OF ORGANIC FARMING AND FLOWER STRIPS AS AGRI-ENVIRONMENT SCHEMES TO ENHANCE THE PRESENCE OF POLLINATORS

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Pollinators have experienced a dramatic decrease worldwide due to agricultural intensification. To counteract the negative impacts of agricultural intensification many countries introduced agri-environment schemes (AES). In our study, we compared the effectiveness in enhancing pollination of

the two most popular AES in Central Germany: organic farming and flowering strips. We selected ten landscapes with triplets of cereal fields: one organic, one conventional with flower strip and one conventional control field. The landscapes were characterized by either small fields or large crop fields. We surveyed pollinators (bees and hoverflies) four times in 2016 and three in 2017. Furthermore, we exposed 50 bumblebee colonies (*Bombus terrestris*, L.) in nine landscapes to monitor their growth and reproduction. We found that the abundance of hoverflies and bees was significantly higher in organic fields and fields with flower strips compared to the control. Furthermore, flower strips supported the highest pollinator abundance and species richness. The ecological contrast in floral resources likely explains these results, and it makes flower strips stand out as the best management supporting pollinators. Bumblebee colonies installed along organic field edges or in flower strips developed better than the control, showing significantly higher weight gains. The combination of waiving pesticides and flower availability probably drove colony development, and the two AES performed equally well. Additionally, at the landscape scale, pollinator responses to AES were moderated by configurational heterogeneity. In fields with flower strips, field size had a negative effect on solitary bee abundance and on bumblebee reproduction, while in organic fields, bumblebee reproduction increased with increasing field size. In conclusion, our results show that organic farming and flower strip schemes support higher pollinator abundances and enable a better development of bumblebee colonies than conventional fields. However, to successfully implement AES, better understanding of pollinator population responses and pesticide effects is needed.

Keywords: Agri-environment schemes, Apidae, flower strips, landscape ecology, organic farming, pollination, Syrphidae

PO288

COMBINED EFFECTS OF POLLEN VIABILITY AND POLLINATOR EFFICIENCY ON SEED SET IN RED CLOVER CULTIVARS

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Clover seed is used in the agricultural sector for animal fodder production and provision of green manure. Availability of organic clover seed is vital for the organic farming sector. However, poor and variable seed set is a persistent problem, particularly among tetraploid red clover cultivars. As clover rarely self-pollinates, pollen transfer by insect pollinators is necessary for good seed set. This in turn, depends on the density and pollination efficiency of pollinators. Long-tongued bumblebees may be most valuable for ensuring a high seed set in red clover, but recent studies have demonstrated dramatic shifts in density and composition of bumblebee communities towards more short-tongued species with higher propensity towards nectar robbing. This could lead to lower pollination rates, especially in tetraploids, which have larger flowers with deeper corolla tubes. In addition, studies suggest that pollen germination and fertilization potential is lower in tetraploid red clover. Pollinator communities will be affected by location and season, and thus there could be a strong interaction between ploidy, geographic locality, and flower phenology, with some cultivars being especially sensitive. In this study we have tried to determine the effects of these factors, including interactions between pollinator community composition and clover cultivar, as well as variation in pollen viability among cultivars and its contribution to low seed set. For different red clover cultivars we investigated floral shape, nectar volume, pollen germination success and pollinator behaviour. Furthermore, we investigated seed set and the amount of pollen deposited on the flower stigma after visitation by 8 different pollinator species with 3 tongue lengths: short (<8mm), medium (8-9 mm) and long (>9 mm).

Keywords: Pollinator, Tongue length, seed set, red clover, bumblebee

PO289

ECOLOGICAL DIVERSITY OF ARTHROPOD FAUNA IN A NATURA 2000 FOREST RESERVE AND NATURAL LANDSCAPE FROM ROMANIA

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The agriculture represent the main important branch of Romanian economy. So far in 1990 the development of this field were based only by the intensive, industrial model in more then 62% from the total of agricultural surface (inclusive field crops and pastures), only little individual farms (under 5-6 ha) had remains private high in the Carpathians mountains or on the subcarpathians hills.This type of agriculture was the result of applying the wrong strategy which has neglected material and energy costs high quality agricultural products as well as the effects on natural capital (the goods and services provided free by the ecosystems). Based on a very diverse richness of type of soils, Romania had a very rich sourse of soil biodiversity especially of the arthropods fillum in some big natural protected reservation. However, studies about biodiversity of soils (in natural or agrosystems) are poor and connected with some ecological peculiar aspects of local, regional areas. The main objective of the study was to present some preliminary information about the soil and ecosystems biodiversity of arthropds from a natural reserve Comana forest from different surroundings natural landscapes. The method used for collecting biological material were soil pit-fall traps which are collecting mobile epigeous arthropods fauna from the ground level and the sampling period was monthly from Mars to November each year. For each ecosystem a number of 25 traps were located on a different number of sampling sites due to the heterogeneity of the environment.Second method was the soil survey of a hole with surface of 25/25/cm and 30 cm deep. All arthropods from that area consisting one sample.The main groups of arthropods are presented from one case study with the values of relative abundance in every type of soil connected with two important drivers: host plant and soil type.

Keywords: Biodiversity, arthropod fauna, conservation

PO290

LANDSCAPE CHANGE IMPACTS ON THE DIVERSITY OF POLLINATORS IN URUGUAY

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Worldwide dramatic land-use changes affect human-insect relationship, pollinators' diversity and reduce the provision of pollination services in agricultural productive landscapes with harsh consequences for human food supply. Managed honeybee hives are often the only solution for farmers to ensure crop pollination. As an example Uruguayan landscapes are currently subjected to expanding afforestation and intensive agriculture, especially soy plantations for the globalized market. Studies on related impacts on insect health and their pollinating services are lacking. We assess impacts of land-use changes on insect pollinator abundance and diversity. We determine interacting pressures on pollinators and underlying processes and mechanisms on landscape scale in order to develop pollinator friendly land-use approaches for a multifunctional, biodiverse and sustainable productive rural landscape. We integrate pollinator specific parameters and evaluation approaches into the existing RuralFutures long term monitoring network and apply standardized data acquisition methods to systematically record data in different land-use types across Uruguay. We generated a database of all appearing Hymenoptera species and present their geographical distribution. Secondly, we established a correlation analysis to measure the association between the appearance of these species and the ground flora within the selected monitoring plots. This relation is being discussed on a land-use level, to create a link between sustainability approaches, landscape heritage conservation and enhancement of regional diversity and local identity in order to develop perspectives on multifunctional productive rural landscapes.

Keywords: Ground flora species richness, land-use change, Pan – Trap method, Hymenoptera diversity, Uruguay, long-term monitoring network RuralFutures, modified *vs* natural landscapes

PO291

THE IMPACT OF TERRESTRIAL OIL POLLUTION IN EVRONA NATURE RESERVE ON ARTHROPODS ASSOCIATED WITH *ACACIA TREES*

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The majority of studies related to oil spill effects have focused primarily on aquatic ecosystems or on their associated coastal ecosystems. However, the effect of oil spills on arid ecosystems has rarely been investigated. Our study aimed to examine the impact of terrestrial oil pollution on the arthropod communities associated with *Acacia trees* in the Evrona Nature Reserve, Israel, where two major oil spills occurred in 1975 and in 2014. Oil exposure may have direct negative effects on arthropods by altering their cuticle or affecting their respiratory system. In addition, it could potentially affect them indirectly via changing the quality of their plant host as habitat or food source. Subsequently, plant quality can have either positive or negative effects on the performance of arthropods along different trophic levels, and this effect may differ among taxonomic groups. We examined arthropod communities on Acacia tortilis trees, a keystone species in the area, in three main sites (two affected by the 2014 oil spill and one by the 1975 oil spill). We sampled the arthropods during August and May of 2016 and 2017, and compared their abundance and composition on oil-polluted *vs* unpolluted trees. We focused on parasitoid wasps —a diverse group with high specificity and sensitivity to environmental changes. Arthropod abundance was lower on trees affected by the 2014 oil spill than on non-polluted control trees, suggesting that polluted trees are less suitable as food source and/or habitat for arthropods. Such a reduction was not apparent in the 1975 oil spill area, suggesting that the negative oil affect is reduced with time. Parasitoid community was diverse including at least 60 morphospecies. However, preliminary results indicated no difference in parasitoid community among polluted and unpolluted trees.

Keywords: Arid ecosystems, terrestrial oil spill, Acacia tree, arthropod community, parasitoid diversity

PO292

SUB-LETHAL EFFECTS OF FUNGICIDE TEBUCONAZOLE ON THE PREDATORY GROUND BEETLE

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Predatory ground beetles on the agricultural landscapes are inevitably exposed to various abiotic stress factors such as fungicides. In this study we investigated, whether and how contact with commercial formulation of a tebuconazole based fungicide over a wide range of concentrations alter the locomotor and feeding activity of the non-target beneficial predatory ground beetles Pterostichus oblongopunctatus. Video tracking of *P. oblongopunctatus* showed that brief exposure to tebuconazole at maximum and minimum field recommended concentrations caused long-term (>24 h) locomotor hypoactivity and hyperactivity, respectively. In addition, tebuconzole affected negatively the feeding activity of the predatory ground beetles. Several hours after fungicide treatment, a significantly remarkable fall in clean food consumption rate occurred in test beetles treated with the minimum and maximum field concentrations of tebuconazole compared to that of the control group. Recovery from locomotion abnormalities and feeding reduction took several days. With this study we show that basic fitness-related behaviours are harmed by the fungicide. All these changes may negatively affect the predation efficacy in agro-ecosystems.

Keywords: Ecotoxicology, locomotor activity, food consumption, Carabids

PO293

EVALUATION OF PARASITOID WASP COMMUNITIES IN THE VINEYARDS OF THE CENTRE-VAL DE LOIRE REGION (FRANCE): IS IT INFLUENCED BY LANDSCAPE STRUCTURE AND/OR AGRICULTURAL PRACTICES?

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Biodiversity is a key factor favoring agricultural sustainability. The presence and action of diverse species of indigenous natural enemies in a cultivated environment are indeed known to provide an important control of crop pest populations and contribute to limit the use of insecticides. In viticulture, the major sector consuming pesticides in agricultural production, an increasing number of actions are developed to enhance arthropod biodiversity and integrate sustainable production. For instance, winegrowers provide habitats for natural enemies by planting hedgerows, grass or flowery strips, they limit the size of their plots, and reduce the work of soils and the use of insecticides. In this context where multiple actions can be conducted, it is important to quantify their relative impacts on the presence of natural enemies. In our study, we focused on parasitoid wasps (Hymenoptera), which can attack a great variety of herbivorous insect species, in several vineyards of the Centre-Val de Loire region in which landscape structure and cultural practices differ. Our first aim is to identify, by molecular barcoding (COI), all the species of parasitoid wasps occurring in 16 different plots spread over the region. Our second objective is to evaluate the influence of landscape structure and/or cultural practices (*i.e.* insecticide use) on parasitoid wasp communities. Over 380 specimens of parasitoid wasps were collected using combi traps (*i.e.* intercepting flying insects) and are analyzed. The results highlight the cultural practices (*i.e.* insecticide treatments) and landscape traits (measured in terms of landscape biodiversity indices via GIS software) favoring the occurrence and diversity of this group of natural enemies. Our work will further allow making recommendations in terms of agro-ecological management in vineyards.

Keywords: Natural enemy, Hymenoptera, DNA barcoding, Landscape, GIS, Vineyards

PO294

CREATION OF A DNA BARCODE LIBRARY FOR ARTHROPODS IN THE VINEYARDS OF THE CENTRE - VAL DE LOIRE REGION (FRANCE)

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The preservation of biodiversity and the enhancement of its associated ecosystem services now represent a worldwide issue, in particular in the context of sustainable agriculture. In France, the "Wine Country", viticulture represents 15% of the value of the national agricultural production and progressively integrates agricultural practices favoring biodiversity, especially in arthropods. Insects, arachnids, myriapods and crustaceae are indeed well known to be reliable indicators of environmental quality as well as major providers of ecosystem services (*e.g.* pollination, pest control). When measuring arthropod biodiversity, the taxonomic identification of collected individuals can be challenging. In the case of molecular identification using metabarcoding, a reference library dedicated to the habitat of interest is required. To our knowledge, no such reference library exists for vineyards in Europe. Our aim is therefore to start building the first reference library of DNA barcodes for vineyard Arthropods via the study of Arthropods in the Centre-Val de Loire Region (France). Specimens were collected using pitfall traps (intercepting arthropods walking on the floor) or combi traps (intercepting flying arthropods) placed in 16 plots. All individuals were geolocated and photographed before DNA extraction. They were then identified using the universal genetic marker of animals, a portion of the cytochrome oxidase 1 (COI) gene, via the platform " Barcode Of Life Data System (BOLD)". In this new reference library of Arthropods, we recorded 297 specimens from 69 families within 15 orders and 5 classes. Among them, 222 have been identified to the genus or the species level (for a total of 131 different species) via BOLD. This library will be open so that results from other studies carried out in vineyards of other regions could be added. Our data set will further permit us to study the role of factors, such as agricultural practices or landscape structure, on arthropod biodiversity.

Keywords: DNA barcoding, COI, Arthropods, Molecular identification, Vineyards

PO295

FLOW OF AQUATIC INSECTS FROM STREAMS TO LAND IN AGRICULTURAL LANDSCAPES

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In agricultural landscapes, riverine ecosystems strongly contribute to the preservation of biodiversity by hosting a large proportion of the local biodiversity. Streams are not isolated ecosystems and they are strongly linked to adjacent terrestrial ecosystems with which, they exchange matter and energy. Streams are primarily considered as passive recipients of matter (nutrients, chemicals) mostly viewed as pollutants, while reciprocal flux has been largely neglected. In this study, we assessed the contribution of emerging aquatic insects to the stream-to-land flow of organic matter and its determinants at local and landscape scale. Using emerging tents, we assessed the influence of local habitat features and landscape characteristics on emerging insect biomass and the relative contributions of the main insect groups (Ephemeroptera, Trichoptera and Chironomidae). We showed large variations in the emerging biomass despite the homogeneity of the monitored agricultural landscape. We found opposed effects of parameters associated to agricultural intensification on Chironomidae abundance compared to Ephemeroptera and Trichoptera abundances. Local habitat parameters were also important with their influence depending on the considered group. However, a positive effect of channel openness on all groups was observed. Using sticky traps, we studied the spreading pattern of aquatic insects in agricultural landscape through dispersal and its temporal dynamics throughout the year. We found large differences in dispersal distance from the streams between insect groups. Most Trichoptera were trapped in the few meters from the riverbank. On the contrary, the abundance of trapped Chironomidae decreased much more smoothly with the distance to the stream. Ephemeroptera exhibited intermediate behaviour. In total, our results showed that local habitat parameters and landscape characteristics strongly influenced the contribution of emerging insects to the flow of organic matter from steam to land in agricultural landscapes, but also its repartition in the landscape through their influence on insect community composition.

Keywords: Agricultural landscape, stream-to-land subsidies, emerging insects

PO296

COLLEMBOLA SPECIES OCCURRING IN HAZELNUT ORCHARDS IN SAMSUN PROVINCE, TURKEY

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Collembola, which are the most abundant terrestrial arthropods, colonise all soil habitats. They play an important role in the decomposition of organic substrates such as leaf litter and hence improve the structure and fertility of soil. Turkey produces 75% of the world's hazelnuts in its Black Sea region. The aim of this study was to determine the collembolan species in hazelnut orchards in Samsun Province. Surveys were done in 41 villages of Atakum, Ayvacik, Carsamba, Salipazarı and Terme Districts in 2014 and 2015. Soil samples to 10-15 cm depth were taken randomly from five sides of each orchard. Bulked 1 kg subsamples from each orchard were processed through Berlese funnels to extract collembolan species. All specimens were preserved in 70% ethyl-alcohol, cleared in lacto-phenol and mounted in Hoyer's medium for identification. A total of twenty one species belonging to 16 genera, namely Ceratophysella, Cryptopygus, Desoria, Folsomia, Folsomides, Isotomurus, Lepidocyrtus, Parisotoma, Protaphorura, Pseudosinella, Sminthurides, Sminthurinus, Sminthurus, Sphaeridia, Tomocerus and Xenyllodes, were identified. *Ceratophysella impedita* Skarzynski, *Xenyllodes armatus* Axelson, *Protaphorura serbica* (Loksa & Bogojevic), *Cryptopygus ponticus* (Stach), *Isotomurus cf. palustris* (Müller), *Pseudosinella cf. sexoculata* Schött, *Lepidocyrtus cf. cyaneus* Tullberg, *Sminthurus multipunctatus* Schäffer, *Sminthurides malmgreni* (Tullberg) and *Sminthurinus elegans* (Fitch) are new records for Turkey.

Keywords: Collembola, springtail, biodiversity, hazelnut, Black Sea region

PO297

CHARACTERIZATION OF INDIGENOUS FIG WASP POLLINATOR POPULATIONS, TIMING OF ADULT EMERGENCE, AND CLEPTOPARASITISM, FOUND IN *FICUS CARICA* IN THE NORTH WEST REGION OF LIBYA

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The study was conducted to identify the indigenous fig wasps that are associated with *F. carica* at the North West region of Libya, to determine their impact on fig production, and pattern of emergence from caprifig syconia. Two species of fig wasps were collected from the inflorescences (syconia) of the caprifig trees; the fig pollinator wasp *Blastophaga psenes* L. (Agaónidae), which represented approximately 99% of wasps collected, and the cleptoparasitic wasp *Philotrypesis caricae* (Pteromalidae) (a first record in the region). The highest densities of *B. psenes* L. recorded were 182 per syconium, while the lowest density was 64 per syconium. The highest density of *P. caricae* occurred at the site with the lowest density of *B. psenes* L., suggesting the cleptoparasite may have had a negative impact on the abundance of the pollinator wasp. Examination of caprifig syconia collected from the field showed most pollinator wasps emerged within the first three days suggesting this may also limit the viability of pollinators within caprifig syconia sold in markets. Nonetheless, a field experiment showed that in 80% of fig syconia pollination, with subsequent maturation of the fig, could be achieved by a single by *B. psenes* L. pollinator wasp.

Keywords: Caprifig, syconium, fig wasp, *Blastophaga psenes* L, Agaónidae, *Philotrypesis caricae*, Pteromalidae.

PO298

CHECKLIST OF TURKISH TRYPHONINAE SPECIES (HYMENOPTERA: ICHNEUMONIDAE)

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In this study, Tryphoninae (Hymenoptera: Ichneumonidae) specimens collected from Thrace Region and Anatolia were evaluated and their identifications were done. All available literature data on Tryphoninae species were reviewed and a checklist was prepared. Among the identified specimens, *Netelia (Netelia) thoracica* (Woldstedt, 1880), *Polyblastus (Polyblastus) pinguis* (Gravenhorst,1820), *Polyblastus (Polyblastus) tuberculatus* Teunissen, 1953, *Tryphon (Stenocrotaphon) obtusator* (Thunberg, 1824) and *Tryphon (Symboethus) heliophilus* Gravenhorst, 1829 were recorded as new species for Turkish fauna. The number of Tryphoninae species in Turkey along with the records presented in this study has reached up to 90.

Keywords: Hymenoptera, Ichneumonidae, Tryphoninae, Checklist, Biodiversity

PO298a

DISENTANGLING FOOD WEBS INVOLVING HENHOUSE-DWELLING MITE COMMUNITIES WITH A FOCUS ON THE POULTRY RED MITES *DERMANYSSUS GALLINAE* AS A PREY: FIRST INSIGHTS FROM LABORATORY PREDATION EXPERIMENTS

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The poultry red mite (PRM) *Dermanyssus gallinae* (de Geer, 1778), is a hematophagous mite associated with birds and is a serious threat to the poultry industry in Europe. Conventionally sprayed treatments against this bioaggressor are largely unsatisfactory. It lives off-host in microhabitats potentially shared by many micropredators, that points it out as an ideal target for biological control by natural enemies inhabiting the poultry environment. This study aimed to characterize the trophic interactions between the poorly-studied indigenous mites with a focus on the predatory activities targeting PRM. We evaluated via *in vitro* bioassays the ability of five putative predatory mite species to feed on PRM mite individuals at different life stages and with different physiological statuses (starved *vs* freshly engorged). Because the level of predation generalism, especially intraguild predation, are expected to influence the suppressive potential of PRM-feeding mites, putative predators were submitted to complementary tests to evaluate their prey preference (choice tests with PRM and alternative mite preys offered). Predation bioassays were conducted with single predatory individuals and potential prey per unit. Four predatory species (*Androlaelaps casalis*, *Macrocheles* sp., *Parasitus fimetorum* and *Cheleytus* sp.) were found to feed on one or more stages of PRM with different prey preferences in terms of mite species. This study constitutes the first step to characterize food webs in mite communities of poultry farm buildings. It highlights the importance of further investigating trophic interactions in poultry farms in order to optimize the natural regulation of PRM by its indigenous natural enemies.

Keywords: Predatory mites, food webs, poultry farms, biological control

Conservation of Saproxylic Insects

PO299

AN OVERVIEW OF THE HABITATS DIRECTIVE SPECIES OF THE MAJELLA NATIONAL PARK (INSECTA)

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The Majella National Park is located at the heart of Central Apennine in the Abruzzo region (Italy), and covers a wide area of about 75,000 hectares. It comprises the Majella massif, the Pizzi - Secine mountain chain, the Morrone mountain chain, and the Orta and Orfento river basins, with elevations ranging from about 400 m up to 2,793 m a.s.l. of Mt. Amaro. In the last two decades several researches have been carried out on different groups of insect, chiefly Coleoptera, Odonata and Lepidoptera, both for deepening knowledges concerning the entomological fauna of the Park, and for providing ecological and geonemic detailed data, in order to assess the most appropriate conservation measures for habitats and threatened species of the protected area. In this framework, specific surveys focusing on the distribution and ecology of the Habitats Directive' species were carried out from 2013 to 2017. As a result of our field and bibliographic researches, 15 species of Insecta listed in the EU Habitats Directive have been detected: four species of Odonata, one of Orthoptera, three of Coleoptera, and seven of Lepidoptera. Among them, the dragonflyes *Oxygastra curtisii*, *Lindenia tetraphylla*, *Coenagrion mercuriale*, and *Cordulegaster trinacriae* are new to the Abruzzo region. The populations of the latter species and of the longhorn beetle *Rosalia alpina* are among the largest discovered in Italy to date. Isolated populations of *Osmoderma eremita*, *L. tetraphylla* and *Coenagrion mercuriale* are particularly threatened, due to the loss, degradation or fragmentation of habitat quality. A detailed geolocalized database has been implemented, and the Standard Data Forms of the five Sites of Community Importance (including the SPA) of the Majella National Park have been updated, since only four species of insects were previously listed, all qualified as DD (Data Deficient).

Keywords: Biodiversity conservation, Insecta, Habitats Directive, Italy

PO299a

SAPROXYLIC BEETLES IN CHESTNUT STANDS OF SOUTHERN APENNINES (CALABRIA): EFFECTS OF FOREST MANAGEMENT ON COLEOPTERA COMMUNITIES

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In managed forests the excessive removal of deadwood often represents a threat to the survival of numerous saproxylic species, with negative effects on the ecological balance of the forest, both in terms of biodiversity and biogeochemical cycles. In the southern Apennines, the impact of deadwood removal on saproxylic species is poorly known. In areas prone to fire risk, the removal of deadwood is envisaged to reduce wildfire risk, which further affect saproxylic species. Our study aims to present a synthesis of the results concerning the monitoring of saproxylic insects in the Calabria Apennines. A monitoring campaign was conducted in three areas of the Aspromonte foothills, in 2017, where chestnut is grown for fruit production and as coppice stands. The results of the sampling of saproxylic beetles belonging to four families (Cerambycidae, Elateridae, Nitidulidae, Oedemeridae,) made by means of windows trap are shown within three typological units of chestnut wood, namely: 1) two coppices at different rotation periods; 2) one chestnut for fruit production in agroforestry setting. A total of 197 species were sampled; 156 in coppice stands and 115 in agroforestry setting. As many as 45 species (23%) were found to be exclusive to a typological unit or a group of structurally similar typological units. Results reveal a rich saproxylic community, including rare and protected species at the national level. The abundance of saproxylic community was favoured in particular by the positive effect of the patchy mosaic of agroforestry system structure of the Apennine chestnut woods. In particular, the managed stands had low amount of deadwood, but with numerous flowering herbaceous plants, which are a fundamental element for the development of saproxylic species. Based on the results, we suggest to limit the removal of deadwood, the conservation of senescent trees, clearings and woodland margins structured with bushes and flowering essences.

Keywords: Saproxylic beetles, chestnut, southern Apennines, forest management.

Insect Species Traits and Their Use in Habitat Management and Conservation

PO300

INSECT-FLOWER INTERACTION NETWORKS VARY AMONG ENDEMIC POLLINATOR TAXA OVER AN ELEVATION GRADIENT

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Interaction networks are sensitive to elevation gradients through changes in local distribution of interacting partners. Here, we use plant-pollinator interaction network metrics to assess the effect of elevation on flowers and flower-visiting insect assemblages on a sentinel mountain used for monitoring climate change in the flower- and insect-rich Cape Floristic Region. We also use these interaction metrics to explain effect of environmental factors on the interaction networks. We did this over four vegetation zones <1640 m a.s.l. Overall, bees were the dominant flower visitors, followed by monkey beetles, and far behind were wasps and flies. The middle elevation zone (650-744 m a.s.l.), which is also an ecotone, had the highest species richness and abundance of interacting plants and insects. Interaction frequency and size of network were also greatest in the middle zone, as were network diversity, generality, and linkage density, while lowest in the peak zone. In sum, there was distinct elevation zoning of flower-visiting insects. The greatest zonal change was between species at the middle compared with peak zone. Large-sized monkey beetles, bees and flies characterized the unique assemblage in the peak zone (1576-1640 m a.s.l.). The insect zonation tracked that of plant assemblages. However, air temperature was the primary driver of bee distribution, with lowest levels in the peak zone. In contrast, monkey beetle distribution was driven mostly by flower assemblages and not by air temperature. We conclude that increased elevation stress from reduced temperatures, changing abiotic weather conditions, and decline in flowering plant composition causes breakdown of bee interaction networks but not that of monkey beetles.

Keywords: Pollinators, bees, monkey beetles, flowering plants, network metrics, elevation stress, altitude

PO301

A BRIEF EVALUATION ON THE SYSTEMATIC AND ECOLOGY OF THE WATER BEETLES (COLEOPTERA: HYDROPHILIDAE, HELOPHORIDAE) IN TURKEY

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Turkey is one of the most diverse areas of the west Palearctic region with its different topographical structure, and forming a natural bridge between Asia, Europe and Africa. Anatolia shows high rates of endemism when the insect variety among living groups is examined. Studies based on aquatic insects are generally focused on certain groups in Anatolian Peninsula. Investigations on the aquatic families of Coleoptera are rather limited compared with others. In the present study recent knowledge about Hydrophilidae and Helophoridae species in Turkey was reviewed with special emphasis on their ecology. Hydrophilidae and Helophoridae represent the largest beetle groups of the superfamily Hydrophiloidea. Up to now, 19 genera, 99 species and 4 subspecies of hydrophilids have been reported from Turkey. The Turkish Helophoridae fauna involves 48 species and 2 subspecies belonging to 7 subgenera. There are many faunistic studies from Eastern Anatolia, Black Sea Region, Central Anatolia and Inner Western Anatolia of Turkey about the mentioned families. However, except a few faunistic records, studies are lacking in the Mediterranean, Thrace, and Marmara regions where there are numerous water sources. Aquatic insects are directly influenced by the physicochemical composition of aquatic habitats and many groups are known to be used as biomonitors. In Turkey, Hydrophilidae and Helophoridae have also been ecologically investigated in the recent years. Studies on ecology of these beetles include habitat preferences, measurements of basic physicochemical parameters of habitats

(temperature, dissolved oxygen, salinity, etc.), and the heavy metal rates of insects. There is still little data from many regions of Turkey, and it is definite that with further studies number of species will be increased.

Keywords: Systematic, Ecology, Hydrophilidae, Helophoridae, Turkey

PO302

SHARE OF ARTHROPODS IN THE DIET OF 3 CARNIVORES THE JACKAL *CANIS AUREUS*, THE GENET *GENETTA GENETTA* AND THE MONGOOSE *HERPESTES ICHNEUMON* IN NORTHERN ALGERIA: COMPARATIVE STUDY

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Food habits of the jackal *Canis aureus*, the genet *Genetta genetta* and the Mongoose *Herpestes ichneumon* were investigated using scat analysis during an annual cycle in the north of Algeria. Our goal through this work is to compare the shares of arthropods taken by these 3 carnivores. This analysis is motivated by the position of Algeria south of the Mediterranean, the presence of a fairly long dry period and a deterioration of increasingly natural habitats. Our results show that the 3 species have a varied diet in which the arthropods participate in the diet with rates varying from 16% in the jackal, 22.31% in the Mongoose and 23.11% in the genet. These are considerably high frequencies; these items thus occupy the second place of each of the schemes. Their consumption is particularly important in summer, many feces are indeed composed in total of these invertebrates were found during the analysis. It is obvious that our work is trying to show a large collection of invertebrates which in fact only provides a modest energy supply to carnivores. This strong presence of arthropods is justified by their ease of capture, thus compensating for their reduced size and thus making the energy balance of the predation positive, especially for carnivores living in habitats subject to strong fluctuations. The ingestion of Arthropods once again explains carnivore plasticity and their euryphagia, they serve as a food supplement of choice, in the absence of essential items. An analysis of the strategies developed by carnivores to mitigate changes in environments is developed.

Keywords: *Canis aureus*, *Genetta genetta*, *Herpestes ichneumon*, diet analysis, arthropods, comparison, Algeria

PO303

REPORT OF PAEDERUS SPECIOUS IN IRAN

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Rove beetles of the genus Paederus cause dermatitis when they come in contact with human skin. Their hemolymph contains a blistering, vesicant and toxic amide named pederin. Dermatitis caused by stimulation of beetle paederus, is a common health problem in Iran especially in Mazandaran (North of Iran) and Fars (South of Iran) provinces. Paederus is a genus of small beetles of the family Staphylinidae (rove beetles) with 622 valid species (Frank and Kanamitsu, 1987). This survey was carried out in several different districts of the Mazandaran and Fars provinces over a period of two years, during spring and summer 2012 and 2013. Rove beetles from selected areas were collected during every visit with the help of aspirator, during day hours (10:00-15:30) with hand catch method using. The greatest number of specimens (334) was collected from north (Mazandaran province) and identified as *Paederus fuscipes*. Other 154 beetles were collected from south (Fars province) were classified in two groups; *Paederus fuscipes* (28.07%) and *Paederus littoralis* (71.93%).

Keywords: *Paederus species*, *Dermatitis linearis*, *Paederus fuscipes*, *Paederus littoralis*, Iran

PO304

THE IMPACT OF ASPECTS OF GARDEN MANAGEMENT ON SOIL MESOFAUNA BIODIVERSITY

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Soil biodiversity is an often overlooked component of global biodiversity, despite being important for supporting soil ecosystem services. As the UK becomes increasingly urbanised, knowledge is required to help make informed garden planting decisions. It has often been assumed that non-native vegetation has negative impacts on biodiversity, however, this hypothesis has not been tested for soil biodiversity. Here the aim was to establish whether the geographical origin of vegetation affected soil mesofauna faunal assemblages, for a UK soil. Traditional taxonomic methods and a molecular phylogenetic approach were used to characterise soil fauna communities of plots planted with vegetation from three geographical regions: ‘Native’, ‘Near native’ and ‘Exotic’. For comparison, additional soil cores were collected from amenity grassland, a lowland heath and a semi-natural woodland. No difference was found either in terms of the taxonomic diversity (1-D & H’) or phylogenetic diversity (PD & MPD), under the different vegetation origin treatments (Native/Near native/Exotic), although differences in abundance were observed. The research suggested that if gardeners are serious about maximising UK soil biodiversity their priority should be lobbying for the preservation of heath and woodland sites, and possibly other less managed habitats. Within the confines of their gardens they should seek to create a heterogeneously structured habitat with a variety of vegetation, ideally with at least the contrast of a flowerbed and a lawn. This work suggests that the vegetation origin ‘Native’, ‘Near native’ or ‘Exotic’ is not of paramount importance for soil biodiversity. The results will be presented within the wider context of the ‘Plants for Bugs’ project which covers the effects of vegetation origin on flower-visiting insects and plant-inhabiting invertebrates. The RHS also has ongoing research into garden gastropod management and the impact some of these methods may be having on soil fauna will be discussed.

Keywords: Garden biodiversity, soil fauna, vegetation origin

PO305

ONE OR TWO SPECIES? MOLECULAR TAXON DELIMITATION OF A NATURA 2000 PROTECTED GROUND BEETLE

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The semi-aquatic, flightless ground beetle *Carabus variolosus* Fabricius, 1787 s.l. is an excellent indicator species of near-natural headwaters in woodlands and brooks. Consequently it has been listed in the Annexes II and IV of the Natura 2000 Directive of the European Union. A few decades ago, the taxon nodulosus Creutzer, 1799 was classified exclusively as a subspecies of variolosus. In recent decades, however, some authors ranked it as a “good” species. This raises the question of whether this is a case of taxonomic inflation or whether the classification of both taxa as species is reasonable. As the species classification based on minor morphological differences between both taxa is still unresolved, carrying serious consequences for conservation purposes as it is not clear whether the populations of nodulosus are protected by the Natura 2000 Directive. In an attempt to resolve this conflict, we employed both nuclear (nuDNA) and mitochondrial DNA (mtDNA) genes. Moreover, we estimated in a numerical taxonomic approach the K2P values for sympatrically occurring Carabus species pairs to define a threshold for the minimal genetic distance which species exhibit within the given genus. The nuDNA shows two well separated lineages which correspond to the two taxa. But a comparable lineage sorting is not visible in the mtDNA sequences. These results give initial evidence for both phylogeography and long-lasting geneflow between the two taxa which are today allopatrically distributed. Our results highlight the role of DNA sequences and numerical taxonomy to solve systematic-taxonomic challenges.

Keywords: Numerical taxonomy, Species delimitation, Biological conservation, Ground beetle

PO306

IDENTIFYING THE OPTIMAL HOST PLANT FEATURES TO PRESERVE AN ENDANGERED INSULAR POPULATION OF *ZERYNTHIA CASSANDRA*

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Zerynthia polyxena is an European butterfly included in the annex IV of the habitat directive since its habitat, where the host plant *Aristolochia* spp. occurs, is declining. The populations inhabiting the Italian peninsula represent an endemic species, *Zerynthia cassandra*. This species also occurs on two islands (Sicily and Elba) and shows there endemic genetic haplotypes. The Elba population is highly threatened, as it inhabits a small area of 4 km^2, frequently prone to fires and only partially included in the National Park of the Tuscan Archipelago. Two species of *Aristolochia* occurs there (*A. rotunda*, *A. lutea*) in different habitats (Mediterranean maquis edges, pine wood edges, abandoned fields, open grasslands). In 2017, we carried out field surveys and rearing experiments to i) define the extent of this population, ii) identify the characteristics of the host plants (vegetative status) and the site characteristics (aspect, insulation, distance from other patches) correlated with the number of eggs occurring on individual plants, iii) compare larval growth and survivorship on the two host plants. The two plants provide an equivalent resource, but insulation, the vegetative status of plants and the occurrence of nearby patches largely explain egg occurrence. These findings allowed to identify the optimal *Aristolochia* patch for egg laying and larval growth. Based on these data, in 2018 we put in place habitat management actions aimed at increasing resource availability for *Z. cassandra* on Elba. These actions were mostly focused in optimizing the insulation of existing *Aristolochia* patches. The comparison between control and experimental patches of 2018 and the comparison with 2017 data allowed to evaluate the effectiveness of the action.

Keywords: *Zerynthia cassandra*, Elba island, *Aristolochia*, habitat suitability, conservation actions

PO307

UNRAVELING THE GORDIAN KNOT OF CONFLICTING HABITAT CORRIDORS: SEMI-OPEN HABITATS AND THEIR BENEFITS FOR HABITAT CONNECTIVITY

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The capacity of insect species to persist in fragmented landscapes depends on movement of individuals between habitat patches which must be sufficient to balance local extinction. Thus to counteract habitat fragmentation, the connectivity of a landscape should be enhanced. Corridors are thought to facilitate movement between disconnected habitat patches. However, corridors simultaneously lead to fragmentation of other habitats. For example, a hedgerow connecting two forest patches through an open habitat forms a barrier for the open land species while serving as a corridor for forest dwellers. To overcome this challenge, semi-open habitats, which contain a mixture of open and woodland habitats, have been proposed, as they may enable simultaneous dispersal of both stenotopic open and woodland species. We studied the potential of semi-open habitats to act simultaneously as corridors for both open and woodland species and assess whether these corridors might be a suitable approach for nature conservation. We sampled low dispersal power carabid beetles in 32 semi-open corridors as well as adjacent open and forest habitats in four regions within Germany encompassing different climatic and edaphic conditions. Effect of semi-open habitat structure on ground beetles abundance was also investigated. Ordination analysis showed a clear separation of the given habitats. Species specific responses to vegetation structure range from avoidance of some patches to an absence of responses and emphasizing the importance of heterogeneity in such habitat. Those results indicate that semi-open corridors are a good strategy for connecting both open and woodland habitats simultaneously.

Keywords: Ecological corridor, Carabidae, semi-open corridor

PO308

MONITORING AND CONSERVATION PROGRAMME OF THE RED WOOD ANT SPECIES *FORMICA PRATENSIS* IN THRACE REGION OF TURKEY

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Industrialization and agricultural activities which have been developing rapidly in Thrace region for the last 50 years have led to accumulation of uncontrolled industrial and urban waste, intensive pesticide use, uncontrolled urbanization, recreational activities and habitat fragmentation in enormous quantities. Undoubtedly, this situation has put a heavy pressure on the species in the region, while some species are no longer seen in the region, populations of some species have fallen drastically. Red wood ant species *Formica pratensis* is unfortunately is among the most affected species. When all nests with faunistic records between 1987 and 2013 were found to be missing, inventory studies was urgently initiated in 2013 and completed at the end of 2014. Distributional patterns, the number of the nests in UTM 5x5 km^2 demonstrated that *F. pratensis*’ conservation status is critically endangered for Turkish national red list. Therefore, following this, the conservation and monitoring program between 2014 and 2017 was integrated into it. Within the conservation program, the nests were surrounded by barbed wire, information boards were hung, and the villagers,

primary and secondary school' students were informed by visiting almost every month. In three years monitoring and conservation studies demonstrated us that only 1/6 of the nests (15 out of 89 nests) have disappeared. These nests deaths are all due to natural causes, no anthropogenic nest damage was observed. This clearly indicates that our monitoring and conservation programme is successful and needs to be carried out for sustainable conservation and management planning.

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Keywords: Monitoring, Conservation, red wood ant, *Formica pratensis*, Turkey.

PO309

IS *VESPA VELUTINA* A TREATH TO WILD BEE COMMUNITIES AND POLLINATION ECOSYSTEM SERVICE?

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Wild bees are the most important insect group able to provide pollination ecosystem service to crops and native plants. In the last decades, several authors highlighted a general drop of wild bees' abundance and species richness due to landscape simplification, use of pesticides and spread of alien species. However, the effect of predation on wild bees' decrease has been marginally explored since now. The yellow-legged hornet *Vespa velutina* Lepeletier 1836, accidentally introduced into France in 2004, is a generalist predator that prey upon a broad spectrum of pollinators such as honeybees and wild bees. In Italy, the hornet is spreading in Liguria and its density follows a gradient along the coastline, increasing from East to West. Because of its diet, it is generally supposed that *V. velutina* predatory behaviour could compromise wild bee communities and consequently pollination services. To test this hypothesis, species abundance and richness of wild bee community has been studied with a pan-trap protocol since 2016 in 6 study areas at different gradient of yellow-legged hornet's density, under the EU funded LIFE STOPVESPA project. Twenty-three monitoring session leads to collect 1,678 specimens and more than 160 wild bee species. The estimate of wild bees richness with rarefaction curves shows high biodiversity in sampled areas. The collected data indicate that some Lasioglossum, Halictus, Ceratina, and Hylaeus species are more likely susceptible to the predation of the yellow-legged hornet because of the overlapping of their fling periods with peaks of *V. velutina* colony populations. The influence of *V. velutina* on wild bees' abundance and richness has been tested with a GLM model. For both response variables, we did not find any significant impact of *V. velutina*. Further surveys are necessary to asses a long-term predation impact.

Keywords: Wild bees, pollination, *Vespa velutina*, predation

PO310

ODONATA COMMUNITY STRUCTURE IN ARTIFICIAL HABITATS

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Several factors act on community structure determining species composition and abundance patterns. Core processes act at local scale (community-level) shaping the observed assemblages like: species' abiotic requirements and species interactions (competition, predation). Artificial habitats (for their simplified structure) are useful models for assessing the main factors affecting community composition and a good proxy to disentangle the assembly rules of the species that inhabit such habitats. Drinking troughs (brickwork tanks) are widespread in Italy and represent a suitable aquatic habitat to be colonized by various taxa. Dragonflies are usually found in drinking troughs and provide an interesting model to study such patterns of assemblage structure. We searched for Odonata communities exhibiting non-random co-occurrence/segregation patterns in drinking troughs in central Italy. We collected 13 Odonata species larvae from 37 sites, built presence/absence matrices, and performed null-model analyses by measuring C-score (co-occurrence index). *Libellula depressa* was the commonest species (21 presences), followed by *Sympetrum sanguineum* (17 presences). We found a pattern of non-random structure for the communities. Interspecific interactions may be the major force driving to observed segregation pattern between species. Focusing on larval-stage, the presence of structured assemblages can be expected due to simplified habitat characterizing drinking troughs that should facilitate larval population growth close to the threshold at which density-dependent interspecific interaction are likely to occur, generating pattern in the very short-term (one year/one season). Interactions within sprawlers (ecological-guild) could be the main driver for the observed total structure, in particular the direct effect of intraguild predation between *Crocothemis erythraea* and Sympetrum spp., that showed high C-scores values in the couple co-occurrence analysis. Overall, intraguild-predation among larvae at different development stages may result in effective segregation pattern, representing a primary process to take into consideration when studying Odonata assembly rules and predict the evolution of the communities in these artificial habitats.

Keywords: Dragonflies, co-occorrence, communty structure, odonata assemblage, articial habitat

PO311

Poster withdrawn.

PO312

Poster withdrawn.

PO313

COMMUNITY ECOLOGY OF AQUATIC INSECTS IN WATER-FILLED TREE HOLES IN THE TROPICAL FORESTS OF THE WESTERN GHATS, INDIA

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Water filled tree holes forming as a permanent or ephimeral freshwater aquatic habitats occupied by an array of invertebrates largely shared by aquatic insects. The baseline data on community diversity and its functionality in these habitats is essential for conservation of this unique habitat. The study was carried out in Silent Valley National Park (SVNP) and New Amaramblam Reserved Forest (NARF) of the Western Ghats, Kerala. The sampling effort comprised of searching for water filled tree holes in the study area. An investigation in 150 tree hole aquatic habitats showed that occurrence of 28 different species with an average of 3-5 species in a tree hole. Most of the recorded organisms (96.8%) belong to order Odonata, Heteroptera, Diptera, Coleoptera, Trichoptera. Altogether, 7505 macrofauna individuals were recorded from 150 tree hole aquatic habitats of SVNP and NARF. Aquatic insects belonging to the orders Coleoptera, Diptera, Trichoptera, Heteroptera, and Odonata together comprised of 96.8% of entire collection. The remaining 3.2% were the representatives of the taxa from Dermaptera, Annelida, Collembola, Gastropoda, Diplopoda, Geckonidae and Anura. Out of 16 aquatic insect taxa recorded, nine were Dipterans, four Coleopterans and one each from order Trichoptera, Heteroptera, and Odonata respectively. About 13 aquatic insect taxa in the tree hole aquatic habitat found in their larval stage and 3 aquatic insect groups such as Hydrophilidae, Dytiscidae of Coleoptera and Heteroptera were found in adult stage. It was found that water filled tree holes which are persistent are having high species diversity and longer food web and tree holes were a perfect analogy to much more complex and larger ecosystems.

Keywords: Tree holes, aquatic insects, The Western Ghats, Trichoptera.

PO314

SOIL MICROARTHROPOD COMMUNITIES IN BARBERA D’ASTI AND BARBERA D’ALBA VINEYARDS

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Among the most widespread cultivars in Piedmont (Italy), La Barbera is known for the quality of its DOCG wines, as well as Nebbiolo and few other ancient local varieties. The aim of this study was to evaluate how different management methods - conventional, IPM, organic, biodynamic – and vineyard longevity impact on soil microarthropods’ community. In soil ecosystem, microarthropods interact with all the other system components and are considered biological regulators. In Piedmont, in the areas of Costigliole d’Asti and Langhe of Alba, 11 study areas were selected. Three soil samples for each site, in the different seasons and vegetative stages, were collected from 2011 to 2013. All arthropod specimens were counted and determined up to the order level. The biological quality of the soil was defined by the ecological index BSQ-ar and by the biodiversity indexes of taxa richness, Shannon, Pielou, Margalef and Simpson. Soil physical and chemical characteristics as soil texture, pH, total organic carbon, total nitrogen, calcium carbonate content were determined. The abundance of arthropods was affected by soil management, soil texture and age of vine plant. The analysis of microarthropod communities by BSQ-ar showed higher values where totally vegetal-based biodynamic cycle is achieved.

Keywords: Sustainable agronomic management, BSQ-ar index, abiotic parameters, biological regulators

PO315

TO WHICH EXTENT *LYMANTRIA DISPAR* L. OUTBREAKS AFFECT SOIL MICROARTHROPODS COMMUNITY: A CASE STUDY IN ELBA ISLAND (ITALY)

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Spring-feeding larvae of *Lymantria dispar* exhibit seasonal outbreaks in temperate forests causing heavy defoliation in host trees. The effect of frass (feces) cover by caterpillar feeding on soil biota is seldom discussed. Microarthropods living in and around soil help the breakdown of plant material and are important indicators of soil health. Mites and collembolans are generally the most represented and known groups; the arthropod community includes also less studied small edaphic orders as Symphyla, Diplura, Protura, Pauropoda, Pseudoscorpions, etc. However, these groups constitute an important portion in biomass and in functional roles. In the Northeastern coastal zone between Nisporto and Procchio of Elba island (Livorno, Italy), the soil microarthropod community was investigated, in March 2016, in three different sites with two closely plots: 1) soil cover by frass pellets after heavy defoliation, 2) natural litter wood cover without any caterpillar frass debris as control. For each plot, five soil cores were sampled; mesofauna was extracted with Berlese-Tullgren funnels. The abundance of main taxa of arthropods were determined and Pearson correlation between groups evaluated. More than 10,000 microarthropods were collected; mites were the most abundant group (73%), followed by collembolans (20%). Acari and Collembola showed higher abundances in control plots. All ‘other arthropods’ were more abundant in attacked wood plots showing differences among taxa (T-test). In particular, microbivores or fluid feeders’ groups were highly represented. Pearson correlation showed that the defoliation action on the tree by *L. dispar* caterpillars affected the food web pyramid. The feeding action of caterpillar plays off against some arthropod groups living in top-layer soil and are decomposers of leaf litter and woody debris.

Keywords: Diplura, Protura, Pauropoda, oak defoliation, soil ecosystem dynamic

PO316

THE COMMUNITY STRUCTURE OF BEETLES IN TWO CROPS FOR BIOGAS PRODUCTION

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The research has been developed in two crop-fields (*Zea mais* and *Arundo donax*) used for biogas production located in the same farm in Milan province. Ten pitfall traps per crops were distributed in field on the base of a hypothetical grid from May 2016 to April 2017 to cover the habitat structure variability. Traps were replaced fortnightly, during the months of May, June, August and December in 2016 and in April in 2017. All specimens were classified to

species level. A total of 8,903 specimens belonging to 12 families of Coleoptera have been detected over the period of observation. Carabidae appeared to be the richest family covering more than 92% of total captures. The differences or similarities in the population sampled have been preliminarily visualized adopting an ordination technique as the Non-Metric Multidimensional Scaling (NMDS). Significant variability among crops and sampling period for the species composition of beetle assemblage was evidenced. Null hypothesis that beetle communities showed the same level of variability was tested by performing a permutational multivariate analysis of variance (Permanova) based on Bray-Curtis similarity coefficient resemble matrix. Finally the homogeneity of the within-group dispersion among groups was performed by a test of homogeneity dispersion (Permdisp). As a result, corn showed a really simplified community, significantly different from that of A. donax. In corn field a few species dominate, producing a really unbalanced species abundance distribution typical of disturbed environment. This is the reason of the strong homogeneity of the corn captures, producing the small cloud of samples in NMDS representation. In A. donax environment, community resulted more diversified, with a very well shaped species abundance distribution, but the remarkable variation of the samples suggests the presence of a still not well established community.

Keywords: *Zea mais*, *Arundo donax*, Carabidae, biodiversity

PO317

METAGENOMIC MITOGENOME ASSEMBLY FROM DUNG BEETLE INTESTINAL CONTENT TOTAL DNA IDENTIFIES MAMMALIAN FAUNA

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Traditional observation-based comprehensive biodiversity measurement is constrained by cost, time and expertise. Therefore, surrogate taxa, representative of wider biodiversity, are commonly used as imperfect ‘proxies’ in biodiversity and ecological studies, which are increasingly benefiting from the combination of high-throughput sequencing and metagenomic methodologies that enable identification of environmental DNA samples. However, it remains unclear whether the combination of surrogate taxa and metagenomics will yield assessments that are rapid, efficient, and accurate. We here tested for the first time the possibility of using the intestinal contents of wild-collected dung beetles (Scarabaeidae) as a source of mammalian DNA, in a proof-of-concept approach to directly detect and identify mammals from savanna-scrub in southern Africa. Dung beetle diversity has been purveyed as an indirect proxy measure of mammalian diversity, owing to dung beetle dependence upon vertebrate dung as a food source, and the ease with which they can be comprehensively sampled compared to vertebrates. Following shotgun sequencing of intestinal content DNA extractions from ten dung beetle species, we used in silico filters to identify mammalian mitochondrial DNA. Seven samples yielded sufficient sequence data to permit the assembly of contigs matching to a blue wildebeest reference mitogenome at a minimum threshold of 10% read coverage. This included four near-complete mitogenomes of two distinct haplotypes, sharing more than 99% sequence identity and assembled at up to 21X mean coverage. Other paired-reads matched mammal mitogenomes held in the GenBank and BOLD databases, and were consistent with wild and domesticated ungulates known from the sampling site. It is conceptually and practically possible to rapidly apply metagenomic techniques to identify mammals upon whose dung the beetles have fed. Since the approach can be readily scaled up, it should be tested in usefulness for assessing population-level genetic diversity, and the detection and identification of rare, endangered, or cryptic mammal species.

Keywords: Biodiversity measurement, Environmental DNA, diet analysis, mitochondrial DNA, scarab beetles, Scarabaeidae, Swaziland

PO318

EVIDENCE FOR RESISTANCE TO MATING DISRUPTION TECHNIQUE IN THE PINK BOLLWORM A PEST OF COTTON

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The pink bollworm, *Pectinophora gossypiella* (Gelechiidae) is a key pest of cotton worldwide. In Israel mating disruption (MD) is used successfully to control the pest in all cotton fields since the early 1990s. However, in recent years a significant increase in the pest population was observed. We studied the hypotheses that: (a) a change in the male’s response to the ratio of sex specific pheromone has evolved, and (b) a change in the population mean in the pheromone amount and/or in its ratio of components have occurred. We tested both hypotheses using an old established population that was never exposed to MD and a newly field collected population that experienced pheromone (MD) environment. We compared the male response to each of the two pheromone components and both components combined. We analyzed females’ glands of the two populations for their amount of pheromone and ratio of its components. In an olfactometer, we tested the male preference to females of their own population *vs* females of the other population and also tested male attactance to females of both populations under clean air and MD environment. We found a significant deviation from the pheromone population mean in the glands of MD experienced females. We also found that although males of the two populations were similarly attracted to the pheromone components, in clean air males preferred females of their own populations. Under MD environment males of both populations were attracted significantly more to MD experienced females. We believe that our results are first to demonstrate the evolution of “resistance” to mating disruption technique due to a deviation of the female produced sex pheromone away of the population mean, as is represented in the synthetic pheromone that is used for MD.

Keywords: Mating disruption, *Pectinophora gossypiella*, cotton, pheromone, resistance, pest

PO319

DRAGONFLY TRAIT DATABASES: WHERE ARE WE NOW?

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There is no doubt that Europe faces an unprecedented decline in insect diversity. Climate change and an ever-increasing anthropogenic influence on most ecosystems increases the need to understand how to mitigate the effects of these influences but also to include the functional links between organisms. The subject of the study of many ecological disciplines is no longer diversity, but “functional diversity”. As a “trait” we refer to a “selective value of organisms” that allow us to understand interactions between organisms and their environment. Since these traits are selected non-randomly, their proper selection and measurement is absolutely crucial. Dragonflies and damselflies are widely used as environmental indicators, but the reality is that we do not have even basic traits for the majority of “well known” species. That is why we have decided to create and maintain an open database of European dragonfly species. Primary information was obtained from books and databases (WOS, Scopus).

However, we still have a pandemic lack of information about many species. That's why we would like to ask you for help. We do not want the individual data to be lost in the sea of averages, and we believe that every piece of information is valuable. For more information, please visit: dragon_database.eu

Keywords: Odonata, indicators, database, insect diversity, functional trait, interactions

PO320

BENTHIC MACROINVERTEBRATES AS THE BIOINDICATORS OF FRESH WATER POLLUTION IN RIVER DORR

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The present study was conducted in River Dorr, district Abbottabad to assess responses of bioindicators towards untreated waste water from Abbottabad city. The sites A and B were studied from January to June 2017. Site A was selected 2km upstream of point source of pollution while site B was selected 2km downstream. Seven taxa of macrobenthos were selected viz., Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Odonata, Diptera and Mollusca. The invertebrates were collected using D-frame kicknet and handpicking. 2205 organisms were collected from site A, belonging to nineteen families. The Ephemeroptera was most diversified and represented by seven families, while Family Rhyacophilidae, Order Trichoptera, was dominant in terms of richness (37.1% individuals). Coleoptera and Odonata, were represented by 0.09% and 1.08% individuals respectively. At site B, a total of 1714 individuals were sampled belonging to eight families. Diptera showed dominance with Family Chironomidae being represented by 67.6% individuals. Coleoptera and Odonata were not encountered at site B at all, while highly pollution sensitive taxon, Plecoptera, was totally absent at both sites. To evaluate degree of pollution, total eleven biological indices were applied including FBI, EPT/C, EPT index, ETO index,%Diptera,%DF, Simpson's diversity index, SIGNAL, CLI, BMWP and ASPT. The overall result of indices regarded site A having excellent aquatic conditions while site B was found to be highly polluted. Functional feeding groups (FFGs) were identified and seven FFG indices were worked out to determine ecological status of the river. The scrappers showed much abundance (55.05%) at site A while gatherer-collectors were dominant (72.8%) at site B downstream. The dominance of certain groups, low richness of predators and absence of shredders revealed ecological disturbances at both sites. The present study revealed that the site A had very good water quality while site B was found to be severely polluted and deteriorated.

Keywords: Benthic macroinvertebrates, Bioindicators, Point source pollution, Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Odonata, Diptera, Mollusca, Family Rhyacophilidae, Family Chironimidae, Biological indices, Functional feeding groups, Scrappers, Gatherer-collectors, Predators, Shredders, Biological monitoring.

PO321

PREDATION PROMOTES DIVERSIFICATION IN MEAN AND VARIANCE OF ANTIPREDATOR TRAITS

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Predators species differ in their hunting style and modes resulting in different selective strength and direction on prey defenses. Thus predation is well known to play an essential role in species traits diversification. Different selection pressures imposed by predators can change the fitness landscape of the antipredator traits. Thus, when prey species are facing changes in the predation regime, this will not only alter the mean of prey's antipredator traits but also might affect trait variance. However, empirical studies on variance change of antipredator traits caused by different predation regimes are scarce. In this study, we present data on change in the mean and variance in spine length and body size of larval abdominal spines in the dragonfly *Leucorrhinia pectoralis*. Larvae occur in either ponds being dominated by predatory fish (fish-lakes) or ponds being dominated by predatory invertebrates (dragonfly-lakes). Thereby, abdominal spines represent a prominent defense against predatory fish but are selected against by predatory invertebrate. Body size is frequently used as a surrogate for fitness. We found that individuals developed longer spines and larger body size in fish lakes than in invertebrate lakes. Moreover, fish-lakes *L. pectoralis* populations had larger variance in spine traits than populations from invertebrate lakes. Our results suggest that change of predation selection pressure will have an impact on both antipredator trait mean and variance. Positive selection from predators will induce higher variance on antipredator traits than negative selection does.

Keywords: *Leucorrhinia pectoralis*; mean and variance; morphology defense; population diversification; predation change

PO322

THERMAL TRAITS OF TRUE BUGS, AN INSECT TAXON WITH HIGH INVASIVE POTENTIAL

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Hemiptera or true bugs are a highly diverse and ubiquitously distributed insect taxon with approximately 42300 species worldwide. After human introduction into a new area, subsequent spread is common. Many species are good flyers capable of handling long distances; eggs and nymphs are translocated with host plants over long distances. Obviously, true bugs have high potential for invasiveness. Temperature is a main parameter for thriving and spread of ectothermic insects. We investigated eight bug species in Europe, distributed over a range of four Köppen-Geiger climate types, from Austria, Bulgaria, Croatia, and Italy, in order to reveal correlations between climate, thermal traits and invasiveness. We assessed the thermal limits (critical thermal maxima and minima, CTmax and CTmin) and thermal tolerance range (TTR) and correlated them with local ambient temperatures (climatological normals, average 1961-1990) and their derivatives (bioclimatic variables). We investigated *e.g. Pyrrhocoris apterus* (native in Austria) which has a wide distribution area from northern Africa to southern Scandinavia and from Western Europe to the west of Siberia and north of China and Pakistan, and *Oxycarenus lavaterae* (invasive in Austria), which was historically mainly found in the Mediterranean Basin including North Africa, and spreads further north into the Benelux countries and Central and Eastern Europe since the 1970s. CTmin ranged from -1.8°C (*Orsillus maculatus*, HR, humid subtropical climate type) to -4.3°C (*Scoloposthetus pictus*, AT, temperate oceanic). CTmax varied from 40.9°C (*S. pictus*, AT) to 48.9°C (*Melanocoryphus albomaculatus*, IT, hot summer mediterranean). TTR ranged from 45.1°C (O. maculatus, HR) to 52.0°C (*M. albomaculatus*, IT). Against our expectations, the insects' thermal traits did not correlate with assessed parameters and bioclimatic variables (except for altitude above sea level). Results show that simple dependencies of thermal traits on ambient temperatures and bioclimatic data seem not sufficient to explain the dispersal success of true bugs.

Keywords: Bugs, thermal traits, climate, invasive insects, Hemiptera

PO323

REGULATION OF NEST AND BODY TEMPERATURE OF *POLISTES BIGLUMIS* AND *POLISTES GALLICUS*, TWO WASPS FROM STRONGLY DIFFERENT CLIMATES

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Polistes is a wide spread taxon with many species in different climate zones. Their relatively small colonies and their open nests make them a perfect object for field studies on thermoregulatory behaviour. We investigated body and nest temperature of an alpine population of *Polistes biglumis* in Austria and a Mediterranean population of *Polistes gallicus* in Italy, in order to elucidate adaptations to local (micro)climate conditions. Both species build their nests outdoors and relatively unprotected. *P. biglumis* nests are found at the east-south-east side of rocks, where they get sun from morning to afternoon, whereas *P. gallicus* often tries to avoid direct solar radiation on the nest. We measured the wasps' body and nest surface temperature by infrared thermography, and the microclimate at the nests with thermocouples and global radiation sensors. The temperatures of two *P. biglumis* and four *P. gallicus* nests and of the wasps were evaluated every ten minutes for whole-day cycles. Results reveal that the maximal temperature of the nests, even at very hot days (Ta ~35-45°C) usually remained below 45°C in both species, increasing up to 50°C only in one *P. gallicus* nest exposed to the sun. This was accomplished by active cooling behaviour via fanning and dispersing of water droplets. The body temperature, at night and in shade, resembled nearly the ambient air temperature in both species. During the day the thorax was more elevated above the ambient in *P. biglumis*. Their thorax never exceeded 40°C, whereas in *P. gallicus* we often measured a thoracic temperature above 40°C (maximum 46°C), because of the extreme ambient temperature and additional exposure to sunshine. Due to the different macro- and microclimate the alpine species seeks the heat via solar radiation, whereas the Mediterranean species tries to avoid additional heat at warm daytimes.

Keywords: Polistes, thermoregulation, microclimate, body temperature, nest temperature

PO324

SOIL BIODIVERSITY OF MICROARTHROPODS AND MACROARTHROPODS (COLEOPTERA: CARABIDAE) IN ARTIFICIAL BLACK PINE STANDS AFTER SELECTIVE SILVICULTURAL THINNINGS

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The Selpibiolife project (LIFE13 BIO/IT/000282) has the main objective to demonstrate the potential of a selective thinning silvicultural treatment to enhance soil biodiversity in black pine stands. The monitoring was carried out comparing selective and traditional thinning methods in artificial and unmanaged stands. The survey took place in the mountain areas of Pratomagno, central Appennines, and Amiata Val D'Orcia (Italy), both before and two years after the silvicultural treatment involving different biotic levels: bacteria, microfungi, macrofungi, ectomycorrhizae, microarthropods, nematodes, macroarthropods (Coleoptera: Carabidae) and flora. The soil microarthropods diversity was assessed by QBS-ar (Soil Biological Quality) whereas ground beetle fauna by biodiversity indices. A total of 16 microarthropod taxa were identified in both sites. The overall results highlighted a different composition of microarthropods and ground beetle communities within the two areas before thinning: springtails were more abundant in Pratomagno, while mites in Amiata. QBS-ar evidenced a more degraded environment in Pratomagno than Amiata. Instead, Carabid fauna comprised 24 species showing higher values of Shannon index in Pratomagno, with the wood degrader *Nebria tibialis subcontracta* as dominant species. In order to explore the ecological linkages between aboveground and belowground biota affecting the ecosystem functioning, a cross-taxon congruence analysis has been carried out before silvicultural operations. The analysis highlighted the absence of a unique factor but rather the mutualistic relationship of all variables, both biotic and abiotic, to regulate the above-below ground subsystems in Pinus nigra plantations. After two years, the silvicultural operation revealed that soil micro- and macro-arthropods were differently affected by treatments. QBS-ar was lower in selective thinning, while ground beetle showed the higher values of Shannon index. More evident and significant results are expected in next years.

Keywords: QBS-ar, Biodiversity indices, Cross-taxon congruence analysis

PO325

THE EFFECT OF THE OCCURRENCE OF *AILANTHUS ALTISSIMA* ON ANT COMMUNITIES: TWO YEARS OF SAMPLING IN A KARST PLATEAU

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The Asian invasive plant *Ailanthus altissima* is one of the major pest in the world, and its adverse effects on plant communities have been documented. In this study, we analyzed the influence of the presence of *A. altissima* on the ant communities inhabiting different habitat types in a karst ecosystem near Trieste, Italy. We analysed three different habitats along a theoretical gradient of complexity: barren land, shrubland and woodland. For each of these habitats, three different sites characterised by the presence of *A. altissima* and three control sites were selected. Ants were collected using pitfall traps, which were left open for two consecutive years. As expected, the three habitat types had different ant communities, but the presence of

A. altissima significantly affected species assemblages within each habitat. Interestingly, species richness was higher in the areas impacted by *A. altissima* than in control areas. This difference is probably due to the effects of *A. altissima* on plant communities, which in turn may affect arthropod communities. On the other hand, the extrafloral nectars produced by *A. altissima* could be an important source of carbohydrates for ants, thus promoting an increment of species richness. Finally, the availability of monthly data on for two consecutive years, allowed to obtain important information on the seasonal changes occurring in ant communities in the study area.

Keywords: Ant community, *Ailanthus altissima*, pitfall traps, karst ecosystem

PO326

BIODIVERSITY OF BUTTERFLY AND THEIR HOST PLANTS IN PATTUNUANG RESORT, SOUTH SULAWESI INDONESIA

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Butterfly conservation area such as Pattunuang Resort in South Sulawesi are gradually being replaced by intensively used farmland especially in lowland and sub-montane regions. The objectives of research were to identify butterfly species and their host plants that serve as nectar/pollen source and larva food plant. A survey with transect methods was conducted in Resort Pattunuang, Maros district, South Sulawesi Indonesia in July to September 2017. The location as a rainfall forest and main part of conservation areas in Bantimurung-Bulusaraung National Park. Based 51 specimens collected, 30 butterflies which significant proportion were Papilionidae, 16 specimens Pieridae, 4 specimens Nymphalidae and 1 specimen Saturniidae. From the seven site observed, 18 (35.3%) butterflies showed preferences at the mating areas, while 12 (23.5) and 6 (11.8%) preferred endemic forest and near the river, respectively. Accumulated butterfly species diversity indices 4 (7.8%) similar number in grass field and sandy soil, but more higher in farming habitat 5 (9.8%). Based function of plants, three butterfly family showed preferences plants as a nectar/pollen source and four family preferred their leaves as a food source. Especially for *Papilio demoleus* and *P. demolion* preferred *Citrus* sp. as nectar and food source for adult and larva. The survey indicated the presence different number of butterfly species based their needs. Further research need a dispersal study based season in order to build a good taxonomic database for Sulawesi butterflies conservation.

Keywords: Sulawesi, Pattunuang Resort, butterflies, biodiversity, nectar, food, conservation

PO327

FOREST AMOUNT POSITIVELY AFFECTS SOCIAL WASP COMMUNITIES AT LOCAL AND LANDSCAPE LEVELS

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The agricultural frontiers are expanding in a very high rate in Neotropics, however, little is known about how forest loss and fragmentation affects insects and its ecosystem services. Social wasps (Vespidae, Polistinae) were used as a model group to investigate how local communities are affect by fragmentation process. We specifically ask: how do local factors (fragment size and vegetation structure) and landscape attributes (forest cover and forest connectivity) affect species richness and abundance of social wasps? We sampled social wasps in 16 Atlantic Forest landscapes across a gradient of forest cover (2.8% to 59.3%), southeast Brazil. Vegetation structure of forest fragments was estimated using a Rapid Ecological Assessment (REA). Moreover, forest cover and forest connectivity were calculated for each landscape using four spatial scales from 250 to 1000 meters (with intervals of 250 m). Using a competing hypothesis and model selection approach we evaluated the contribution of explanatory variables on local wasp communities. We captured 672 specimens representing 32 species within 11 genera. Species richness and abundance were positively associated with both forest cover and fragment size, while forest connectivity and vegetation structure were not relevant. These results suggest that social wasps strongly depend on forest amount, but most species are not forest specialists. Social wasps are predatory insects which probably use forest fragments as nesting habitats and agroecosystems as feeding habitats where wasps can find high densities of preys (crop pests). Therefore, forest restoration in crop-dominated landscapes is important from both biodiversity conservation and sustainable agriculture perspectives.

Keywords: Agricultural landscapes, Biodiversity conservation, Habitat fragmentation, Social insects, Tropical forests.

PO328

TWO DECADES OF A DRAMATIC CHANGE IN THE ARTHROPOD COMMUNITY OF THE DEAD SEA REGION

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The Dead Sea region in Israel is characterized by arid habitats, salt wetlands, and green oases. The geological past, the geomorphological activity, and the extreme aridity and warm climate of the region have created unique ecosystems. Decades of intensive human disturbance in the region have led to tremendous changes in the landscape and characteristics of the natural habitats; in turn leading to significant changes in their flora and fauna composition. The particular characteristics of the Arthropods make them a useful tool for identifying and characterizing changes in the ecosystem. Extensive sampling of terrestrial Arthropods was carried out in the region from 2014-2018. The community structure of the recent species assemblage was compared to the rich collection data of the last 100 years. Eighteen species of ants new to the region were recently found along the Dead Sea shores, five of which are known invasive species from tropical countries. In Einot Tzukim, a unique National Park to the north of the Dead Sea, a generalist ant (*Camponotus fellah*) was introduced into the park in only the last two decades, but has since become established and common at the most disturbed sites in the park, and possibly competes with the related endemic ant (*Camponotus kugleri*); while *Megacephala euphratica*, the largest Cicindelid beetle in Israel, adapted to hyper-saline habitats, has disappeared from all but one site in the Dead Sea region; and the endemic mole cricket *Gryllotalpa marismortui* has not been seen for 20 years. In this study we demonstrate the rapid change that has taken place in the Arthropod assemblage of different taxonomic groups, resulting from dramatic changes in the environment. We also suggest that rare species might offer an effective tool for identifying ecological processes and phenomena.

Keywords: Dead Sea region, Arthropod community, Habitat loss, Habitat assessment

PO329

AN ANNOTATED LIST OF INSECTS, ASSOCIATED WITH CUPRESSACEAE FAMILY IN BULGARIA

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The representatives of Cupressaceae are often used in private garden design and urban landscape design because of their high ornamental value and plenty of cultivars. Over the last twenty years these plants have been massively cultivated in Bulgaria and become very popular. Most members of the family have alien origin and the only native representatives that grow wild in Bulgaria are some junipers - *Juniperus communis*, *J. excelsa*, *J. oxycedrus* and *J. sabina*. The cypress family in Europe is attacked by several insect pests. They may cause significant damages to ornamental plants and reduce their aesthetic and market value. Other insects affect only plant reproduction, feeding on reproductive organs and seeds. The aim of this work was to summarize the information of reported insects, associated with Cupressaceae in Bulgaria. The annotated list of country`s detected insects includes data on their organotrophic specialization, established localities, host plants and the authors who published the records. It is writen on the basis of available literary sources. Part of the information was specified after personal communication with the authors, reported the insects. The annotated list consists of fifty-six species, belonged to five orders and nineteen families. For thirty-three of them trophic relationships with family Cupressaceae in Bulgaria are established. According to literature data, other twenty-three species also develop on Cupressaceae, but reports of their feeding on these plants are absent in Bulgaria. Three of the discussed insects are restricted to greenhouses. Fourteen of them feed on cones and seeds – thus suspected to affect natural regeneration in wild habitats, seventeen on leaves, shoots and branches, and twenty-two are xylophagous insects. The most numerous are representatives of order Coleoptera. There are no records about these insects as pests and the damage they cause in Bulgaria, except for genus Carulaspis and Gelechia senticetella.

Keywords: Cupressaceae, Bulgaria, insects

PO330

LEPIDOPTERA TORTRICIDAE FROM ETHIOPIA

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Lepidoptera Tortricidae of Ethiopia are still very little known and since the Meyrick (1932) publication of the results of expedition to Abyssinia in the years 1926-1927 only the below discussed collections were made. The history of the entomological expeditions of the University of Molise to southeast Ethiopia in the years 2009 and 2010 were described by Razowski & Trematerra (2010). Those of two expeditions to the Oromia Region, by the entomologists of the Molise University and University of Milano, were mentioned by same authors (Razowski & Trematerra, 2012). A more recent expedition of University of Molise was realized on February-March 2017 to Amhara Region and Oromia Region in Central-Northeast Ethiopia (Razowski, Trematerra and Colacci, 2018). During the six expeditions were visited different localities and habitats, in particular the itinerary of the expedition were: • Southeast Ethiopia - in April 2009 (Omo Valley, 1400 m/a.s.l., and Wenchi Crater, 2900 m/a.s.l.); November 2009 and February 2010 (Bale Mountains, from 1600 to 3100 m/a.s.l.); • Southwester Ethiopia - in November 2010 and January 2012 (Didessa River, 1280 m/a.s.l., Dabeda River, 1800 m/a.s.l., and Mute Forest, 2060 m/a.s.l.); • Central-Northeast Ethiopia - in February-March 2017 (Suba Forest, 2500 m/a.s.l., Zegie Peninsula, 1820 m/a.s.l., and Ambo Park, 2170 m/a.s.l.). In all cases the *Lepidoptera Tortricidae* were collected as adults, both by entomological net during the day and by light trap at night-time. During the expeditions a total of 63 species of Tortricidae were identified of which 34 taxa were described as new for the first time. Of the latter new species, 2 species belong to the tribe Tortricini and 5 to the tribe Archipini (subfamily Tortricinae); 7 specie belong to the tribe Bactrini, 1 to the tribe Olethreutini, 1 to the tribe Enarmoniini, 6 to the tribe Eucosmini and 11 to the tribe Grapholitini (subfamily Olethreutinae).

Keywords: Ethiopia, *Lepidoptera Tortricidae*, faunistic records, new species

PO331

ARE MOTH COMMUNITIES MORE STRONGLY SHAPED BY HABITAT-SCALE OR LANDSCAPE-SCALE DRIVERS?

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Most remaining near-natural habitats nowadays exist as isolated fragments embedded in an anthropogenically modified landscape. Heterogeneity of the surrounding landscape as well as the quality of habitats inside conservation areas are known to affect the assembly of local communities. Landscape configuration may even override the role of local drivers of ecological communities. Understanding the complex interplay between these two spatial classes of drivers is key for increasing the effectiveness of conservation management in small isolated nature reserves. We studied species-rich communities of one group of mobile insects (viz. nocturnal moths) in two isolated nature reserves, Pineta san Vitale (PSV) and Pineta di Classe (PDC) near Ravenna (NE Italy). Our goal was to assess whether local or regional drivers are more important in shaping local moth communities. Between 2015 and 2017, we sampled moths using automated light traps at 30 downy oak forest sites within each park. With this sampling designed we aimed to cover as much as possible the site-to-site variation that exists within both nature reserves. At each light trap site multiple abiotic factors (soil, climate) were measured. We also recorded species composition and structure of the vegetation. Finally, we determined landscape configuration and land-use around each trap site in buffers of 400 to 2000 m diameter. Evaluation of moth samples (more than 15000 individuals, representing over 200 species) is still under way. Overall, both nature reserves revealed a relatively similar vegetation (PDC: 163 vascular plant species; PSV: 164 species) and the moth species pools were largely shared between them. Shrub heterogeneity between trap sites was higher in PDC whereas herb heterogeneity and functional divergence was higher in PSV. These data will now be used to assess whether local habitat factors or landscape composition around the trap sites more strongly shape alpha, beta and gamma diversity of moth communities.

Keywords: Moth community, Biodiversity, landscape composition, small-scaled ecological changes

PO332

HIGH ALTITUDE PAPILIONOIDEA (LEPIDOPTERA) OF TSAGHKUNYATC MOUNTAIN IN ARMENIA

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In this study, the altitudinal distribution of butterflies of Tsaghkunyatc mountain in Armenia was given. Butterflies were collected from March to October 2005- 2017. 129 species were found, checklist is given. This revealed that the relationship between the total numbers of butterfly species and individuals and altitude is not well prominent hump-shaped. A significant relationship was also recorded between the altitude and the abundance and richness. However, the diversity and evenness index yielded a semi-constant relation in altitude because of existence a good few species at high altitudes. Therefore, the information obtained is indispensable for habitat management generally and for planning of Protected Areas in particular.

Keywords: Abundance, Armenia, evenness, Diversity, Papilionoidea, Species richness.

Session 7.
Genetics and Evolutionary Biology

Evolutionary Genomics

PO333

GENETIC DIVERSITY AND PHYLOGENY AMONG *VESTALIS LUCTUOSA* POPULATIONS ON JAVA ISLAND USING RAPD MARKER

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Vestalis luctuosa is a zygoptera that found mostly in mountain streams. deforestation threatens the presence of this dragonfly. The study of the genetic diversity of this damselflies is still little studied. RAPD is a popular molecular method used to study genetic variations primarily at the population level. The purpose of this study was to know the genetic diversity and phylogeny among *Vestalis luctuosa* populations. Polymorphism result from electrophoresis visualization was analyzed using NTSYS PC V22. The results of the analysis indicate the influence of geographical location to the phylogeny distance between population.

Keywords: *Vestalis luctuosa*, RAPD, Polymorphism

PO334

PHYLOGENETIC DISTRIBUTION OF REPETITIVE DNA SEQUENCES IN PARASITOID HYMENOPTERA

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Two important classes of the repetitive DNA sequences, namely, telomeric repeats and clusters of the ribosomal DNA (rDNA), were studied in parasitoid Hymenoptera using fluorescence *in situ* hybridization (FISH). These studies demonstrate that the number of rDNA sites in parasitic wasps usually varies from one to six per haploid karyotype. Since this parameter was found to generally correspond to the chromosome number, and the latter value often decreases in advanced parasitoids, so does the number of rDNA clusters. However, certain exceptions to this rule, *e.g.* varying numbers of rDNA sites in different members of the same genus sometimes having the same chromosome number, as in chalcid wasps of the genera Eurytoma (Eurytomidae) and Trichogramma (Trichogrammatidae) with n=5-7 and 5 respectively, also occur. Moreover, we have recently found that multiple CG-enriched chromosome segments which usually correspond to rDNA clusters in parasitoid Hymenoptera, are characteristic of all chromosomes of another chalcid wasp, *Trichospilus diatraeae* (Eulophidae) with n=7. Our views on the phylogenetic distribution of the TTAGG telomeric repeat in the order Hymenoptera also have dramatically changed during the last years. Specifically, the (TTAGG)n telomeric motif was initially considered characteristic of the Hymenoptera in general, but it was actually found only within the families Formicidae and Apidae (Aculeata). Furthermore, we failed to detect the TTAGG repeat on telomeres of all studied parasitoids of the superfamilies Ichneumonoidea, Cynipoidea and Chalcidoidea. In addition, all other main groups of aculeate Hymenoptera were also shown to lack this motif. On the other hand, our recent finding of the TTAGG telomeric repeat in a few members of the family Tenthredinidae (Symphyta) suggests an ancestral nature of this motif in the order Hymenoptera in general as well as its subsequent loss somewhere within the clade Unicalcarida and independent reappearance in the Apidae and Formicidae.

Keywords: Parasitoid Hymenoptera, parasitic wasps, rDNA, TTAGG telomeric repeat, FISH, phylogeny

PO335

HAPLOTYPE DIVERSITY OF THE BLACK CHERRY APHID *MYZUS CERASI* (FABRICUS, 1775) (HEMIPTERA:APHIDIDAE) BASED ON THE MITOCHONDRIAL CYTOCHROME OXIDASE I SEQUENCE

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Myzus cerasi (Fabricus) is a substantial agricultural pest causing leaf curling and transmitting several non-persistent viruses through indirect damage. In this research, adressed the analysis of mitochondrial cytochrome oxidase I (COI) gene sequences from twenty five Turkish populations of black cherry aphid collected from *Prunus avium*, and one population from Greece. Phylogenetic trees were constructed using Maximum Likelihood, Minimum evolution tree, Neighbor joining and the Unweighed Pair Group Method of Aritmetic Averages (UPGMA). *Myzus persicae* (Sulzer) and *Myzus borealis* Ossiannilson were included as out-groups. Four haplotypes were identified based on nucleotide substitutions and the mean intraspecific divergence was calculated to be% 0.3. Some populations diverged from the rest of the groups and form a clade (Haplotype B) with *Myzus borealis*. The rest of the haplotype diversity comprises Haplotype A and Haplotype C with individuals characterized as *Myzus cerasi* pruniavium and Haplotype D with *Myzus cerasi cerasi*. It was concluded that M. cerasi diverge into two subspecies and it must be reevaluated whether this pest is monophagous or oligophagous in terms of plant type dependence. This finding contributes to taxonomic researches and pest management strategies through DNA barcoding.

Keywords: Black cherry aphid, cytochrome oxidase I (COI), haplotype diversity, phylogenetic tree

PO336

INSECTBASE: A RESOURCE FOR INSECT GENOMES AND TRANSCRIPTOMES

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The genomes and transcriptomes of hundreds of insects have been sequenced. However, insect community lacks an integrated, up-to-date collection of insect gene data. Here, we introduce the first release of InsectBase, available online at http://www.insect-genome.com. The database encompasses 138 insect genomes, 116 insect transcriptomes, 61 insect gene sets, 36 gene families of 60 insects, 7,544 miRNAs of 69 insects, 96,925 piRNAs of *Drosophila melanogaster* and *Chilo suppressalis*, 2,439 lncRNA of *Nilaparvata lugens*, 22,536 pathways of 78 insects, 678,881 untranslated regions (UTR) of 84 insects and 160,905 coding sequences (CDS) of 70 insects. This release contains over 12 million sequences and provides search functionality, a BLAST server, GBrowse, insect pathway construction, a Facebook-like network for the insect community (iFacebook), and phylogenetic analysis of selected genes.

Keywords: Insect genome; Database; InsectBase

PO337

MICRORNA EVOLUTION AND THE ORIGIN OF INSECTA

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The monophyly of Hexapoda and the relationships between four hexapod lineages (Protura, Collembola, Diplura and Insecta) failed to be resolved well based on morphological and gene sequence data. MicroRNAs (miRNAs) have been proved as new effective phylogenetic markers, but have not been studied in basal hexapods. Here, we newly sequenced small RNAs from 7 entognathous hexapod groups, retrieved known miRNAs from miRBase, and predicted miRNAs from published genome data of arthropods. In a total, 91 conserved miRNA families from 36 panarthropod taxa were analyzed, and the tree constructed using the presence/absence matrix of miRNA families in Arthropoda is mostly consistent with the popular phylogenetic relationships of arthropods. New miRNA families are continually added to almost each major clade of arthropods, and two conserved miRNAs are newly found to be originated in hexapods, in favor of the monophyly of Hexapoda. In addition, four conserved miRNA families exist specifically in Diplura and insects, which strongly support Diplura as the closest extant relative of insects.

Keywords: miRNA evolution, molecular phylogeny, Protua, Collembola, Diplura

PO338

TANDEM DUPLICATION OF TWO TRNA GENES IN THE MITOCHONDRIAL GENOME OF *TAGIADES VAJUNA* (LEPIDOPTERA: HESPERIIDAE)

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To explore the debated phylogenetic relationship of two Hesperiidae subfamilies, Pyrginae and Eudaminae, and contribute to the understanding of the evolution of mitogenomic architecture in butterflies, we sequenced the complete mitogenome of *Tagiades vajuna*. The mitogenome is a typical circular duplex molecule of 15,359 bp. Apart from the standard 22 tRNAs, it has a tandem duplication of trnS(AGN) and trnE, which is unique in lepidopteran insects. Comparison with *Ctenoptilum vasava* indicates that the trnS1 duplication is not an ancestral state shared with other species of Tagiadini. Independent origin of the trnS1duplications was further confirmed by the reconstruction of the ancestral character state based on the topology of the phylogram. Furthermore, comparative analysis of mitogenomes with and without tRNA duplications indicates that tRNA duplication does not alter the codon usage pattern. The mitogenome has negative AT- and GC-skews, and it is highly A+T-biased (79.7%). The AT-rich (or control) region (283 bp) contains “ATAGA” and “ATTTA” motifs. Regarding the phylogenetic analysis, we found that removal of the third codon position (3CP) from datasets used for the mitochondrial phylogenomics of Hesperiidae is likely to produce results that are more consistent: Pyrginae were rendered paraphyletic by Eudaminae in both analyses of the dataset from which the 3CP was removed (13 PCGs+all RNAs), but inclusion of the 3CP resulte d in a destabilized topology, resulting in both monophyly and polyphyly. We conclude that shallow-phylogenies of insects should pay close attention to compositional and mutational biases in mitogenomes.

Key words: Lepidoptera, Hesperiidae, phylogenomics

PO339

CONDITION ALTERS THE DEGREE OF SEX-BIASED GENE EXPRESSION

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In most species, both sexes display a vast array of morphological, physiological and behavioral differences. One of the theories developed to explain the evolution of sexual dimorphism provides that females choose their mates on the basis of male sexual display traits, in order to increase the chances that their own genes are successfully transmitted to the generations beyond. Thus, male sexual display traits have to show “condition dependent expression”, working as indicators of male genetic quality, since only individuals with a good overall condition can afford their expression. However, with exception of the heterogametic chromosomes, females and males are genetically identical. Thus, differences are likely to be generated by differential expression of their common genome. Here, we analyzed different Drosophila RNA-seq datasets in which individuals’ condition has been manipulated in different ways. Results suggest that a reduction in condition alters the pattern of expression of sex-biased genes, shifting the transcription profile of males and females towards a more sexually intermediate phenotype.

Keywords: Drosophila, Sexual dimorphism, RNA-seq

PO340

ORTHOLOGY-BASED GENOME ANNOTATION AND INTERPRETATION

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The OrthoDB [Evgeny M. Zdobnov *et al.*, 2016] catalog of orthologs, www.orthodb.org, represents a comprehensive resource of comparative genomics data to help researchers make the most of their newly-sequenced genomes. It classifies protein-coding genes from available genomes into groups of orthologs descended from a single gene of the last common ancestor (LCA) of each clade of species. The hierarchical orthology classification obtained by applying the clustering procedure at each major radiation point of the considered species phylogeny allows the user to select the most relevant level. OrthoDB collates available gene functional information from UniProt, InterPro, GO, OMIM, model organism phenotypes and COG functional categories, and provides evolutionary annotations including rates of ortholog sequence divergence, gene copy-number profiles, homology-related sibling groups and gene architectures. The latest OrthoDB release (OrthoDB v9.1) covers more than 5000 species, including almost 200 vertebrates, ~150 arthropods, ~300 fungi, ~3800 bacteria and now includes also plants, archaea and viruses. The set of almost 150 arthropod species offers the most comprehensive orthology resource for arthropod comparative genomics. Comprehensive and unbiased comparative analyses rely on the completeness of the analysed genomes. However the assessment of the quality of the genome assemblies is often complicated, and mostly limited to technical measures such as scaffold/contig N50. OrthoDB’s sets of Benchmarking Universal Single-Copy Orthologs, BUSCO

http://busco.ezlab.org, provide a rich source of data to assess the quality and completeness of genome assemblies and their gene annotations. OrthoDB and BUSCO enable improved and extended orthology-based genome annotation and interpretation in a comparative genomics framework that incorporates the rapidly growing numbers of sequenced arthropod genomes, thus providing powerful tools that can facilitate arthropod genomics.

Keywords: Orthology, OrthoDB, BUSCO, Single-Copy Orthologs, arthropod genomics

PO341

GENOMIC INSIGHTS ON THE BIOGEOGRAPHY OF THE TIGER MOSQUITO, *AEDES ALBOPICTUS*, IN ITALY

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The Asian tiger mosquito, *Aedes (Stegomyia) albopictus*, is one of the most invasive animal species, having spread in a few decades from its original range in South-East Asia all over the world, thanks to its high ecological plasticity and ability to exploit human mediated transportation. This has a significant public health impact, being the species a competent vector of numerous exotic arboviruses also in temperate areas, as testified by the 2017 Chikungunya outbreak in central and southern Italy, with almost 500 human cases. Understanding the biogeography and the dynamics of this extraordinary invasion process is therefore not only of scientific interest but could also provide insights on epidemiologically relevant factors, such as susceptibility to insecticides used for control interventions and for which differences between populations have been reported. In addition, a better knowledge on the invasion history of the tiger mosquito could help preventing further invasion processes. In order to investigate population structure and invasion history of *Ae. albopictus* in Europe, we genotyped by double digest Restriction site - Associated DNA sequencing (ddRAD) more than 100,000 SNPs for 9 populations across Italy, 1 from Greece and 1 from Albania. To identify the most possible origin(s) of the Italian populations, we concatenated our data with a published worldwide reference panel of 20 populations, including samples from both, the native and the invasive range of *Ae. albopictus*. Results show important genetic differences between the European countries included in the study and suggest a pattern of multiple invasions from at least two or three different source populations. High genetic intra-population diversity among Italian populations supports large propagule pressure, with genetic drift and admixture among different source populations creating a complex genomic pattern with generally low levels of differentiation and signs of Isolation By Distance among the Italian populations.

Keywords: *Aedes albopictus*, invasion history, population genomics, ddRAD-sequencing

PO342

Poster withdrawn.

PO343

MASSIVE-SCALE DATA INTEGRATION FOR THE STUDY OF INSECT EVOLUTION

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Modern-day genomics has provided us with an enormous amount of data, providing new-found opportunities for evolutionary genomics. However, this is easier said than done. While the field of bioinformatics moves towards integrating massive amounts of data from various sources, there a few challenges even for seasoned bioinformaticians: 1) Existing data are often hard to find and reproduce (*e.g.* lacking meta-data); 2) Data integration on a massive scale requires extensive, specialized knowledge; 3) The results of the data integration studies are often rather abstract, making biological interpretation difficult. While it is currently often challenging for every-day biologists to overcome these hurdles, it is the biological knowledge of these same experts that is required to develop well-informed hypotheses and interpretations given these data. Therefore, we aim to make this massive-scale data integration easy-to-do and common practice. Our framework provides three main concepts that should make the challenges easier to overcome: store all data in a FAIR (Findable Accessible Interoperable and Reusable) way, provide easy and on-the-spot cross-querying of all stored data, and allow the user to explore the data with simple visualizations that link the integrated data to the biology, *e.g.* drawing statistical summary graphs and overlaying different types of data on biological pathways. We provide the above concept by utilizing modern database concepts, integrating existing databases, and providing a framework of underlying programming able to query the data both in abstract and visual ways while the biologist only has to utilize the user-friendly overlying framework. Finally, with the above in mind we will provide an example on how to utilize this concept of data integration, where we aim to find cross-species evolutionary features to classify genes into multiple functionally-related categories, and to predict new gene-members of prominent biological pathways or processes.

Keywords: Data integration, evolution, bioinformatics, databases, omics

PO344

ANOPHELES GENOME ASSEMBLY IMPROVEMENTS GUIDED BY EVOLUTION

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Results from the Anopheles 16 genomes project and other initiatives have made publically available the genome assemblies of more than 20 anophelines. In contrast to the highly contiguous and near-completely chromosomally mapped *Anopheles gambiae* PEST genome assembly, the initial draft assemblies of the more recently sequenced anophelines vary greatly in their contiguity and only a handful have at least partial chromosomal assignments. Ongoing assembly improvement strategies including generating additional sequencing and/or physical mapping data continue to improve these initial drafts, *e.g.* 98% of the highly contiguous *Anopheles albimanus* assembly is now anchored to chromosomes. Here we employ four different computational approaches that each attempt to characterise evolutionary signatures from analysing the conservation of gene order - synteny - to identify putative scaffold adjacencies in the current assemblies. Leveraging information from cross-species comparisons enables the delineation of well-maintained synteny blocks, which provide support for scaffold adjacencies that reconstruct the conserved arrangements in assemblies where orthologues are located at scaffold extremities. These predictions can be validated through comparisons with independent evidence, where available, such as physical mapping data, or mapped RNAseq-derived transcripts that link scaffold extremities, or indeed *de novo* assemblies from new sequencing data. The consensus predictions offer evolutionarily well-supported sets of scaffold adjacencies that lead to the improved

contiguity of draft assemblies without the associated costs or time investments required for additional sequence-based support. Thus, whether employed as supporting data for experimentally based assembly improvement approaches, or as complementary data to further enhance such improvements, or as stand-alone evidence as part of an assembly-building pipeline, these evolutionarily guided approaches offer a handy new set of utensils in the genome assembly toolbox.

Keywords: Anopheles, genome assembly, genome synteny, orthology, comparative genomics, bioinformatics

PO345

DISTRIBUTION OF PLANT CELL WALL DEGRADING ENZYMES IN BEETLES OF THE FAMILY CERAMBYCIDAE

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Beetles of the family Cerambycidae represent the most diverse group of xylophagous insects. Larvae of this family of beetles live in a challenging environment. In order to develop, they have to consume woody tissues which contain large amounts of plant cell walls. These structures are mainly composed of polysaccharides recalcitrant to decomposition such as cellulose, hemicelluloses and pectins. Previous studies have shown that the genome of beetles of the superfamilies Chrysomeloidea (which includes the Cerambycidae) and Curculionoidea contain gene families encoding so-called plant cell wall degrading enzymes (PCWDEs). The products of these genes enable these beetles to break down most polysaccharides of plant cell walls. The Cerambycidae is an understudied group of beetles from a molecular point of view. Therefore, to expand our knowledge on the evolutionary history and functional characteristics of PCWDEs in cerambycid beetles, we sequenced, using RNA-Seq, midgut transcriptomes of 22 species representing six out of the eight subfamilies of this family of beetles. Our sequencing effort provides the most extensive genomic/transcriptomic dataset for this group of insects to date and will lead to novel insights into the biology of the Cerambycidae. The annotation of all the carbohydrate-active enzymes (CAZymes) present in these 22 transcriptomes will allow us to identify gene families encoding putative PCWDEs and follow their distribution in these beetles. Phylogenetic analyses will provide insights into how these gene families evolved during the radiation of this group of xylophagous insects.

Keywords: Cerambycidae, PCWDEs

PO346

THE WHOLE GENOME DNA METHYLOME AND ITS REGULATORY FUNCTIONS IN *TRIBOLIUM CASTANEUM*

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DNA cytosine methylation is a vital epigenetic regulator of eukaryotic development. Whether this epigenetic modification occurs in *Tribolium castaneum* has been controversial, its distribution pattern, establish mechanism, functional mechanism and biological functions have not been established. Here, using bisulphite sequencing (BS-Seq), we first confirmed the existence of DNA methylation and described the methylation profiles of the four life stages of *T. castaneum*, then we identified that both symmetrical CpG and non-CpG methylcytosines were observed in the *T. castaneum* genome. While, asymmetrical non-CpG methylation was predominant in the methylome, which was strongly concentrated in intergenic regions and introns but absent from exons. Gene body methylation was negatively correlated with gene expression levels. Symmetrical CpG methylation, which occupied a small part in *T. castaneum* methylome, was primarily enriched in gene bodies and was positively correlated with gene expression levels. How does the novel DNA methylation pattern establish in *T. castaneum*? then we identified the DNA methyltransferases and methyl-CpG-binding domain protein and clarify the establishment mechanisms, functionl methanisms and biological functions of DNA methylation in *T. castaneum*.

Keywords: *Tribolium castaneum*, DNA methylation, Development, DNA methyltransferase, methyl-CpG-binding domain protein

PO347

GENE DUPLICATIONS IN THE PEA APHID GENOME: TOWARDS SUB- OR NEO-FUNCTIONALIZATION?

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Gene duplications are considered to be one of the drivers for the acquisition of new cellular and developmental functions during evolution. The pea aphid genome contains a large number of gene families with duplicated members. In this paper, we describe these duplicated families. A phylogenomic approach seeded in the pea aphid genome and including 6 other aphid genomes indicates that 1,834 gene families are monophyletic and duplicated specifically in the pea aphid, and totaling 5,300 different proteins. Forty families have more than 10 genes. The families with 2 paralogs are the most frequent with 1,175 pairs and 2,350 proteins. Those expansions are not enriched in particular function, based on GO categories. In order to test the hypothesis of sub- or neo-functionalization of some of these duplicates, we select a subset of duplicated genes that have single-copy orthologs in at least two other aphid genomes: 770 families are selected (1,926 proteins). We have compared the mRNA profiles of these genes across 19 different tissues and/or developmental conditions and obtain a ranked list of pairs of genes according to their profile similarity. Using a binary model to compare profiles we show that 332 pairs of genes have identical profiles whereas using a non-binary model lowers this number an order of magnitude (35). These mRNA expressions will be correlated with the accessibility of the chromatin (from FAIRE-Seq data) of the corresponding loci, to test the hypothesis that duplicated genes are or are not in different chromatin states. Ultimately, we are analyzing the protein evolution parameters of these duplicated genes to search for traces of positive selection within them. This integrative approach should indicate whether high level of gene duplication in the pea aphid genome is related to acquisition of new functions, in the perspective of the capacity of aphids to develop phenotypic plasticity.

Keywords: Genome evolution, *Acyrthosiphon pisum*, phylogenomics

PO348

A MALE-SPECIFIC GENOME REGION CONTAINING THE SEX DETERMINATION SWITCH IN *AEDES AEGYPTI*

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Aedes aegypti is the principal vector of several important arboviruses. Among the methods of vector control to limit transmission of disease are genetic strategies that involve the release of sterile or genetically modified non-biting males, which has generated interest in manipulating mosquito sex ratios. Sex determination in *Ae. aegypti* is controlled by a non-recombining Y chromosome-like region called the M locus, yet characterisation of this locus has been thwarted by the repetitive nature of the genome. In 2015, an M locus gene named Nix was identified that displays the qualities of a sex determination switch. With the use of a whole-genome BAC library, we amplified and sequenced a ~200kb region containing this male-determining gene. We show that Nix is comprised of two exons separated by a 99kb intron, making it an unusually large gene. The intron sequence is highly repetitive and exhibits features in common with old Y chromosomes, and we speculate that the lack of recombination at the M locus has allowed the expansion of repeats in a manner characteristic of a sex-limited chromosome, in accordance with proposed models of sex chromosome evolution in insects. The sequenced region has a high degree of similarity to the corresponding section of the M locus in the updated reference genome assembly, AaegL5. Further evidence from sequencing technologies like 10X linked reads and RNA-Seq may help to identify features involved in the suppression of recombination and the evolution of sex determination, and yield insights that can be applied to increase the efficiency of genetic strategies for vector control.

Keywords: M locus, Sex determination, Chromosome evolution, Genomics, BAC, PacBio

PO349

EVALUATION OF POLYMORPHISM IN FIELD POPULATIONS OF *THRIPS TABACI* (THYSANOPTERA, THRIPIDAE) OF ONION CROPS

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Thrips tabaci Lind. is one of the most harmful insect pest of onion crops worldwide and has recently attracted special interest as the first identified vector of TSWV and IYSV, two economic important tospoviruses. Moreover, this species shows considerable variations in biological and ecological traits within and between populations. Knowledge of the relative influence of population-*versus* species-level genetic variation is important to understand patterns of phenotypic variation and the ecological relationships that exist within tabaci species complex. This study reports preliminary results on the genetic variability checked through the analysis of the mitochondrial cytochrome c oxidase subunit I gene (COI), in field populations of *T. tabaci* sampled on onion crops in a large grown area of the Falerna coastline (Calabria Region, South Italy). Each specimen thrips was placed in a 0.5 ml tube with 100 µl of lysis buffer. The samples were homogenized using a mixer mill and then, were incubated at 95°C for 20 minutes. Specific primers Ron and N2353 were used in order to amplify a portion of the COI region, under the following cycling conditions: 15 min initial denaturation, followed by of 40s denaturation at 95°C, ramp to 60°C in 1 min, hold for 5s, 2 min extension at 72°C. The PCR products were purified over silica columns (Quiagen) and a sequencing reaction with 20 ng of DNA was performed, through an Applied Biosystems automated sequencer 3130 xl. The COI sequences were edited by eye with Bioedit 7.2.5, virtually translated into the corresponding amino acid chain (to detect frame-shift mutations and stop codons), aligned, and used for a preliminary phylogenetic reconstruction. Performed analyses have allowed to delineate the *T. tabaci* populations infesting onions in the investigated areas through the evaluation both of the genetic diversity and of the phylogenetic relationships existing between them.

Keywords: *Thrips tabaci*, polymorphism, South Italy, species complex

PO350

EXPLORING THE FUNCTION AND DOSAGE COMPENSATION OF SEX-CHROMOSOMES IN THE DIAMONDBACK MOTH (*PLUTELLA XYLOSTELLA*) WITH LONG-READ GENOME SEQUENCING AND RNA-SEQ

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The Lepidoptera have a female heterogametic sex-chromosome system with most extant species having a ZZ/ZW sex-chromosome constitution. This relatively unusual feature has important consequences for the evolution and maintenance of sex chromosomes, including dosage compensation. Recent studies have suggested that the pattern of dosage compensation is highly conserved across the Lepidoptera and that reproductive tissues were a confounding factor in early experiments. Using RNA-seq data from sexed somatic and gonad tissue and a long-read genome assembly we find that this hypothesis appears to be supported in *P. xylostella*. Producing a PacBio reference genome for this species was challenging due to the very small size of *P. xylostella*, we mitigated this issue by using a combination of pooled sequencing and redundant allele resolution. The final assembly was integrated with single chromosome sequencing data to identify the female-specific W-chromosome and a published RAD-seq linkage map to assign the remaining sequence to chromosomal positions. Exploring the content of the female-specific W chromosome reveals a putative feminising element and its flanking sequences suggests that the activity of transposable elements may be responsible for its evolutionary origin. Reanalysis of a previously published W-chromosome sequence suggests a similar locus may also be present in Mediterranean flour moth *Ephestia kuehniella*. The prevailing hypothesis for the non-canonical origin of the lepidopteran W chromosome is discussed in the context of our data in addition to the potential implications for the evolution of the sex-determination cascade.

Keywords: Lepidoptera, sex chromosome, sex determination, dosage compensation, *Plutella xylostella*, PacBio, genome assembly, RNA-seq,

PO351

THE GENOME OF ENDOPARASITOID *COTESIA CHILONIS* (HYMENOPTERA: BRACONIDAE)

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Cotesia chilonis (Hymenoptera: Braconidae) is the major larvae endoparasitoid wasp of striped stem borer, *Chilo suppressalis* (Lepidoptera: Crambidae), one of the most economically important rice pests in Asia, Northern Africa, and Southern Europe. The natural parasitism rates range from 10% to 30% and may be as high as 90%. This wasp is regarded as one of the most important natural enemies of *C. suppressalis*, and has great potential for pest biological control. Here, we present the draft genome of this endoparasitoid wasp. Adopting a whole-genome shotgun sequence strategy, we generated about 64.44 Gb of Illumina reads and 7.63 Gb of Pacbio reads. The filtered reads were assembled using ABySS to yield a 189.53 Mb assembly with an Contig N50 size and Scaffold N50 size of 1.22 Mb and 2.21 Mb, respectively. The GC content of the genome is 30.36%. The genome assembly is of high quality, as 237 out of 248 core eukaryotic genes mapping approach (CEGMA) genes are complete in the assembly; and 93% of 843 Single-Copy BUSCOs are full in length. We used Optimized Maker-based Insect Genome Annotation (OMIGA) pipeline to identify the protein coding genes and after integrating the evidences of RNA-Seq, *de novo* prediction and homolog protein alignment, an official gene set (OGS) of 14,142 genes of *C. chilonis* was obtained. We identified 16 small nucleolar RNA (snoRNA), 39 small nuclear RNA (snRNA), 144 transfer RNA (tRNA), 148 ribosome RNA (rRNA) and 111 microRNA (miRNA). We identified gene families with apparent expansions or contractions in *C. chilonis* by a comparison with other ten Hymenoptera insect genomes, and observed some gene families associated with parasitic behavior were significantly expanded in *C. chilonis*.

Keywords: *Cotesia chilonis*, genome, gene families, parasitic behavior, comparative genomics

PO352

THE GENOME OF WASP *MACROCENTRUS CINGULUM* PROVIDES NEW INSIGHTS INTO POLYEMBRYONY AND IMMUNE EVASION

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Background: Parasitoid wasps are well-known natural enemies of major agricultural pests and arthropod borne diseases. The parasitoid wasp *Macrocentrus cingulum* has been widely used to control the notorious insect pests *Ostrinia furnacalis* (Asian Corn Borer) and *O. nubilalis* (European corn borer). One striking phenomenon exhibited by *M. cingulum* is polyembryony, the formation of multiple genetically identical offspring from a single zygote. Moreover, *M. cingulum* employs a passive parasitic strategy by preventing the host's immune system from recognizing the embryo as a foreign body. Thus, the embryos evade the host's immune system and are not encapsulated by host hemocytes. Unfortunately, the mechanism of both polyembryony and immune evasion remains largely unknown. Results: We report the genome of the parasitoid wasp *M. cingulum*. Comparative genomics analysis of *M. cingulum* and other five hymenopterans, two dipterans, two lepidopterans, one coleopteran, one hemipteran, and one mite species were conducted, finding some gene families with apparent expansion or contraction which might be linked to the parasitic behaviors or polyembryony of *M. cingulum*. Moreover, we present evidence that the microRNA miR-14b regulates the polyembryonic development of *M. cingulum* by targeting the c-Myc Promoter-binding Protein 1 (MBP-1), histone-lysine N-methyltransferase 2E (KMT2E) and segmentation protein Runt. In addition, Hemomucin, an O-glycosylated transmembrane protein, protects the wasp larvae from being encapsulated by host hemocytes. Motif and domain analysis showed that only the hemomucin in two endoparasitoids, *M. cingulum* and *Venturia canescens*, possessing the ability of passive immune evasion have intact mucin domain and have similar O-glycosylation patterns, indicating that the hemomucin is a key factor modulating the immune evasion. Conclusions: We provided the draft genome of an endoparasitic wasp *M. cingulum*. The microRNA miR-14b participates in the regulation of polyembryonic development, and the O-glycosylation of the mucin domain in the hemomucin confers the passive immune evasion in this wasp. These key findings provide new insights into the polyembryony and immune evasion.

Keywords: *M. cingulum*, genome, polyembryony, immune evasion, comparative genomics.

PO353

AFFINITIES AND DIVERGENCE OF AEDES MOSQUITOES USING BAYESIAN RELAXED CLOCKS AND COMPLEX MODELS OF EVOLUTION

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A detailed understanding of Aedes evolutionary affinities is important for at least three reasons: 1) it guides proper taxonomic classification of genus and species; 2) it aids planning comparative genomics by defining the sister-taxa of medical relevant species; 3) it provides a framework for estimating divergence and reconstructing paleo-ecological scenarios. Current knowledge of Aedes phylogeny and divergence is still based on fragmented molecular matrices and has never been explored using complex Bayesian inferences and relaxed clocks. Here we have blasted public databases and assembled a multi-gene alignment composed of mitochondrial coded COI, COII, NADH4, 16S and nuclear coded 28S, ITS, 18S, enolase and arginine kinase for a total of ~7500 nucleotide positions of 145 species, 100 Aedes species, 30 species from other Culicidae genera and 15 species from the Syrphidae family used to root the trees. Bayesian inferences indicate a poor resolution at the single gene level, while concatenation has higher resolution power; we compared our phylogenies using homogeneous and heterogeneous replacement models to define nodes that are susceptible to systematic errors. Our phylogenies revealed *A. falvopictus* and *A. japonicus* as the sister species of respectively *A. albopictus* and *A. koreicus* two species of growing interest in Europe. Our divergence estimates based on Bayesian relaxed clock reveal a strong among-genes heterogeneity of the evolutionary process, but overall indicate the origin of the Aedini tribe in the late Triassic and his radiation in the early Jurassic. Our study is the first attempt to provide a multi gene phylogeny of Aedes using heterogeneous replacement models and relaxed clock estimate of their divergence: results are relevant for clarifying some long lasting systematic issue and for providing a pleo-ecological scenario which may increase our understanding of Aedes biology and epidemiology.

Keywords: Mosquito, Phylogeny, Aedes, Clock

Evolution and Development of Functional Traits

PO354

UNVEILING THE MOLECULAR GROUNDS OF AGGRESSIVE MIMICRY IN THE MYRMECOPHILOUS APHID *PARACLETUS CIMICIFORMIS*

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Aphids (Hemiptera:Aphididae) are characterized by complex life cycles developing different alternative morphs depending on environmental variables such as photoperiod, temperature, host plant or food quality. Within aphids, members of tribe Fordini (Eriosomatinae) exhibit particularly complex life-cycles often including myrmecophilous morphs. Species of this tribe alternate between a gall-dwelling phase on *Pistacia* sp. and a root-dwelling phase on different Poaceae. In most species of Fordini, morphs of the root-dwelling stablish myrmecophilic interactions with different ant species. Most of these interactions fall well within the typical trophobiotic type. However, we recently described a different aphid-ant interaction between the aphid *Paracletus cimiciformis* and ants of genus Tetramorium. Differently from other Fordini, *P. cimiciformis* was shown to produce to alternative wingless root-dwelling morphs instead of a single one. One of these morphs (the so called green or round morph) shows a typical trophobiotic interaction with Tetramorium ants while the alternative one (the so called, white or flat morph) exhibits aggressive mimicry towards the same ants. Being both morphs produced through parthenogenesis (*i.e.* they are clonal) they are genetically identical so that both morphological and behavioural differences between them should result from differential gene expression. Aiming at understanding the differences at the molecular level between the round and flat morphs of *P. cimiciformis*, especially those that are behind the unique behaviour exhibited by the white morph, we conducted an RNAseq survey to compare the transcriptomes of both morphs. Up to about 700 and 600 transcripts were found to be upregulated in the round (green) and flat (white) morphs respectively. Apart from global comparisons, results from a detailed analysis of some of the differentially expressed genes are discussed.

Keywords: aphids, transcriptome, phenotypic plasticity, myrmecophily

PO355

GENETIC DISSECTION OF REPRODUCTIVE SUCCESS TRAITS OF THE PARASITOID *C. TYPHAE*

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Studying mechanisms that drive parasitoids adaptation to their host is interesting in the double perspective of understanding co-evolution processes and for their efficiency and environmental safety in biocontrol programs. In this context, we study the ability of two strains of *Cotesia typhae*, an African parasitoid specialized on the crop pest *Sesamia nonagrioides*, to reproduce in an allopatric host population from France. On this host, the two *C. typhae* strains differ significantly on several reproductive traits such as parasitism success and offspring size. To investigate the genetic bases underlying these phenotypic differences, we developed QTL approach based on Restriction site Associated DNA markers (RAD-tags), a high throughput marker technique. Crosses between homozygote individuals from the two strains were performed to generate F2 and F8 recombinants. Taking benefit from the haplo-diploid reproduction of *C. typhae*, crosses were designed to produce clonal sibships females from recombinant males. More than 4000 individuals from 92 F2 and 91 F8 clonal sibships were phenotyped for the mentioned traits and the 183 clonal sibships were sequenced. To maximize confidence in clonal sibships genotypes inference, *de novo* and reference based (using a draft genome of *C. sesamiae*) approaches were performed with STACKS software. Stringent selection of loci was then conducted to build a high quality genetic map. The 550 selected loci are organized in 10 linkage groups corresponding to the ten chromosomes of *C. typhae*. Identification of QTL associated with the phenotypic traits characterized is ongoing.

Keywords: Parasitoid, reproductive success traits, QTL, RAD-seq

PO356

LOCOMOTOR ACTIVITY ASSOCIATED TO A POINT MUTATION OF A MOTH FORAGING GENE

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A kinase dependent on cGMP, called PKG I or cGK I, is known for its conserved function in food-related behaviors of several insect species. The gene coding for this kinase is called foraging in these species. In the Mediterranean corn borer, *Sesamia nonagrioides* Lefèvre (Lepidoptera, Noctuidae), we discovered two Snfor alleles differing by several synonymous single nucleotidic polymorphism (SNP), but a single non-synonymous SNP, meaning one amino acid change in the protein sequence, glycine *vs* serine. Larvae having the serine allele move more frequently between two food patches in an actimeter than those having the glycine allele. Since this phenotype is also associated to a difference in the for gene expression, we isolated the effect solely due to the mutation by analyzing heterologous expression in Drosophila. We generated two transgenic lines, each carrying one of the moth allele, using the UAS/Gal system. We checked that the transgenic lines expressed their respective transgene. Registering the flies' locomotor activity in the actimeter revealed that individuals having the serine transgene moved more than those having the glycine transgene. These results strengthen the hypothesis of a causal link between the point mutation and the associated phenotype. The amino acid change being in the catalytic domain of the kinase, it may modify its affinity for its substrates. S. nonagrioides is found on both wild and cultivated plants that may exert different selective pressure on feeding activity. We discuss the adaptive character of the mutation from first results on the influence of host plant and geography on the allelic distribution.

Keywords: Crop pest, behaviour, gene, mutation, genotype-phenotype relationship, adaptation

PO357

REDEFINING REPRODUCTIVE DORMANCY IN DROSOPHILA: A PHYSIOLOGICAL AND GENETIC STUDY

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Organisms regularly encounter unfavorable conditions and the genetic adaptations facilitating survival have been of long-standing interest to evolutionary biologists. Winter is one particularly stressful condition for insects, during which they encounter low temperatures and scarcity of food. Despite dormancy being a well-studied adaptation to facilitate overwintering, there is still considerable controversy about the distribution of dormancy among natural populations and between species in Drosophila. The current definition of dormancy as developmental arrest of oogenesis at the previtellogenic stage (stage 7) distinguishes dormancy from general stress related block of oogenesis at early vitellogenic stages (stages 8 – 9). In an attempt to resolve this, we scrutinized reproductive dormancy in *D. melanogaster* and *D. simulans*. We show that dormancy shows the same hallmarks of arrest of oogenesis at stage 9, as described for other stressors and propose a new classification for dormancy. While we found significant differences between individuals and the two Drosophila species in their sensitivity to cold temperature stress, we also noted that extreme temperature stress (8°C) resulted in very strong dormancy incidence, which strongly reduced the differences seen at less extreme temperatures. Applying this modified classification, we show that both species express dormancy in cosmopolitan and African populations, further supporting that dormancy uses an ancestral pathway induced by environmental stress. We further investigated the underlying genetic basis of dormancy in the Drosophila Genetic Reference Panel by applying our modified classification and GWAS. We conclude that dormancy in Drosophila should not be considered a special trait, but is better understood as a generic stress response occurring at low temperatures.

Keywords: Dormancy, Drosophila, stress response, oogenesis, degeneration, GWAS

PO358

BUTTERFLIES IN DIRE STRAITS – ECOLOGICAL CONSTRAINTS AND FUNCTIONAL TRAITS EXPLAIN HIGH GENETIC DIFFERENTIATION BETWEEN SICILY AND ITALIAN PENINSULA

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Genetic lineages expand over continents following environmental changes, but when they meet frequently form narrow areas of overlap. Understanding the mechanisms maintaining the boundaries between lineages requires to relate genetic diversification, functional traits and dispersal constraints. We analysed for the mitochondrial COI gene 1249 specimens representative of the butterfly communities (82 species) occurring around the narrow Messina strait (3 km wide) separating Sicily from the Italian Peninsula and we compared them with 4680 specimens belonging to the Palaearctic region. A 40% of species showed a high genetic differentiation across the strait (Gst>0.5) and a 32.4% revealed Sicilian endemic lineages. Several Sicilian lineages (41.7%) had their closest relatives in distant Palaearctic areas, instead of the closest Italian Peninsula. For each species, we evaluated traits determining: i) mobility, feeding ecology, phenology, eco-physiological constraints, ii) dispersal costs based on the length of direct paths and on environmental permeability and iii) the impediment for dispersal represented by winds blowing over the strait during the flight period. Phylogenetic regressions showed that haplotype diversity and non-standardized population differentiation (Dst) are mostly related to intrinsic phenological traits, while standardized differentiation (Gst) was also explained by local constraints (winds and climatic costs of dispersal). In conclusion, only matrilines of more dispersive butterflies seem to cross the strait and admix over it given the unfavourable winds and did not evolve endemic variants. Understanding the determinants of local genetic diversification required the integration of genetic sequences, functional traits and physical constraints obtained for an entire community at continental scale.

Keywords: COI differentiation, Messina strait, butterflies, functional traits, environmental constraints, dispersal

Evolutionary Ecology

PO359

THE ADAPTIVE VALUE OF MALE GUARDING IN THE LIFE OF A SPIDER MITE WITH FIRST MALE PRECEDENCE

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Life-history reproductive decisions, such as mating strategies and offspring sex allocation have large impact on the fitness of individuals. In the spider mite *Tetranychus urticae* first male precedence ensures that only the first male mating with a focal female sires her offspring and thus males often guard juvenile quiescent females until they molt. But males are also frequently found mating with adult females, even at the risk of they being already mated. Males are then faced with the option of either investing time in guarding quiescent females and thus assuring paternity or mating with sexually mature females, but risking not producing offspring with these mates. Here we first investigate the adaptive value of each of these strategies, under different life-history conditions. In particular, we look at the effect of sex allocation in shaping the adaptive value of guarding juveniles *versus* searching for adult females. We find that: 1) coexistence of both strategies is possible, and 2) guarding is favoured by a male-biased primary sex ratio and larger female maturation times, and disfavoured by an increase in the mortality rates of the population. We support these findings with a set of foward simulations illustrating how guarding will evolve under explicit demographic conditions such as degree of first male precedence, primary sex ratio, mortality rate, female maturity time and meeting rates. By studying a wide but biologically plausible parameter space, we aim to understand the conditions necessary for premate guarding to evolve.

Keywords: Reproductive strategies, sexual selection, male precedence, haplodiploidy, evolutionary dynamics.

PO360

THE DISTRIBUTION PATTERN OF WOLBACHIA IN INSECTS

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Many arthropods, especially insects, are infected with inherited endosymbiotic bacteria of the Wolbachia genus. These symbionts are ordinarily transmitted vertically in maternal lineage of the host, which could lead to coevolution of the host mtDNA and symbionts. The reasons of extraordinary symbiont expansion among insects, are not yet clear. However, the components of this remarkable success obviously include the abilities of Wolbachia to induce reproductive abnormalities that increase the frequency of infected individuals, to increase the fitness of the infected host, and to be sporadically transmitted between non-related species. Wolbachia symbionts are classified into about twenty phylogenetic supergroups, with two of them being most often found in insects. Here we try to reconstruct the pattern of Wolbachia distribution in insects. The symbiont incidence and the symbiont genetics are considered for families and orders of insects. Original data include results of Wolbachia surveys in different families of butterflies, moths, ants, bees, wasps, mosquitoes, flies, fleas, bugs, grasshoppers, locusts and others. No Wolbachia infection has been found in some taxa that we assume could be explained by ecological features of these groups. Specific Wolbachia genetic contents have been revealed for many taxa that indicate non-random symbiont distribution among insects.
The research is supported by Russian Foundation for Basic Research, grant # 16-04-00980.

Keywords: Wolbachia, insects, genetics

PO361

PHASES OF POPULATION CYCLE AND MTDNA PATTERN OF *LYMATRIA DISPAR* (LINNAEUS, 1758) IN WESTERN SIBERIA POPULATIONS

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Forest pest *Lymantria dispar* (Lepidoptera: Erebidae) is a typical eruptive species that is characterized by rotation of peak population and population collapse. Moth populations of West Siberian Plain of temperate forests have a population cycle with period of 7-9 year. A ‘travelling wave’ phenomenon is observed for this area in the direction from west to east. The reason(s) for this phenomenon is not yet clear. Here we studied the mtDNA pattern of populations that were at different phases of the population cycle. Our aims were i) to find genetic evidence of actual moth migration with the moving of the outbreak front, ii) to determine boundaries of populations, and iii) to reveal effect the travelling wave on mtDNA pattern of moth populations. In the presentation we demonstrate the results of this study.
The research is supported by Russian Scientific Foundation, grant # 17-46-07002.

Keywords: *Lymantria dispar*, mtDNA, population, West Siberia

PO362

MOLECULAR DETERMINANTS OF HOST CHOICE IN SPIDER-WASPS (HYMENOPTERA: POMPILIDAE)

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Spider wasps (Pompilidae) are solitary, predatory aculeates that exclusively hunt, sting, and paralyze spiders (Araneae) to use as provisions for their own ectoparasitic larvae. Most pompilid wasps are prey generalists that utilize spider taxa belonging to the large araneid “RTA-clade” (named after the retrolateral tibial apophysis present in males). However, some phylogenetically derived pompilid taxa are prey specialists that target specific spider groups such as wolf spiders (Lycosidae), orb weavers (Araneidae), crevice weavers (Filistatidae), or tarantulas and trapdoor spiders (Mygalomorphae). Additionally, one large cosmopolitan clade of spiders, the Haplogynae, is not known to be utilized by pompilid wasps at all. While prey choice must partly depend on practical factors such as prey availability and size, these explanations cannot fully account for the existence of pompilid taxa that narrowly specialize on (or avoid) particular clades of spiders. The main paralytic components of pompilid wasp venoms are pompilidotoxins (PMTXs): short peptides of 13 amino acids that either slow or prevent inactivation of the voltage-gated sodium channel by binding to the same site targeted by certain scorpion and cnidaria toxins. Mass spectrometry of pompilid wasp venoms has uncovered at least two different PMTX analogues with different potencies, while sequencing of the toxin binding site in sodium channels from diverse spider taxa reveals certain mutations unique to arachnids, and others unique to haplogynes. Through site-directed mutagenesis experiments, PMTX sensitivity is known to be altered by amino acid replacements that either reverse or remove the charge of sodium channel residues at the PMTX binding site, or that introduce amino acids with short hydrophobic side chains. Based on these data and on models of PMTX binding, it is proposed that taxon-specific prey choice and avoidance in pompilid wasps is tied to the effectiveness of different PMTX analogues in inactivating spider sodium channels, which show diversity at critical toxin binding sites that may have evolved under natural selection favoring PMTX resistance.

Keywords: Hymenoptera, Solitary Wasps, Pompilidae, Parasites, Evolution, Phylogeny, Predator-prey relationships, Spiders, Spider-wasps, Molecular Adaptation, Venom, Neurotoxins, Pompilidotoxin, PMTX, Voltage-gated sodium channel

PO363

SUBCELLULAR CADMIUM COMPARTMENTATION IN *SPODOPTERA EXIGUA* LARVAE FROM CONTROL AND CADMIUM STRAINS

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Cadmium sequestration mechanisms may be important in metal tolerance response of herbivores pests. The aim of this study was to establish the distribution of Cd in different subcellular fractions in *Spodoptera exigua* larvae dependently on animal origin from cadmium strains (the larvae from cadmium strains after metal selection lasted 15, 70 and 158 generations) and control strains. We measured the concentration of Cd subcellular fractions in the whole animals, but with guts cleaned off food contents. The following fractions were obtain in differential centrifugation procedure: granules, cellular debris, microsomes and lysosomes, heat stable proteins (containing metallotionein) and heat denaturable proteins. Total amounts of Cd were higher in animals from control strains than from cadmium strain. And, we stated differences in cadmium localization in most examined cellular fractions between animals from cadmium and control strains. Summarizing, it seems that changes in participation of compartments contained: lysosomes and microsomes, heat shock proteins and heat denaturable proteins may be important in better cadmium tolerance in case of the cadmium strains larvae.
The study has been financed by the National Science Centre Poland, grant no 2016/21/B/NZ8/00831

Keywords: Cadmium selection, cellular compartments, Lepidoptera, larvae

PO364

EVOLUTION OF HOLOMETABOLOUS INSECTS

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Insects with complete metamorphosis (Holometabola) are the most successful group on earth. Complete metamorphosis radically transforms the insect body, leading to distinct larval and adult stages separated by a pupa. Why the pupa evolved, remains unclear. One barely studied explanation is the decoupling of growth annd differentiation in Holometabola. The larva growths and most differentiation occurs in the pupa. By reconstructing the holometabolous ´Eve´, I studied the relationship of rapid growth and the evolution of complete metamorphosis. I compiled a dataset on insect growth comprising of 350 species. Due to a wide variety of growth measurements, I calculated growth ratios for 15 of these species. My preliminary results show no correlation between growth and holometabolism. But I could show higher growth rates and higher variance in growth rates in egg size corrected data in Holometabola. However, my dataset builds a well-documented basis to further investigate the decoupling of growth and differentiation in insects with complete metamorphosis.

Keywords: Complete metamorphosis, growth, ancestral reconstruction, evolution

PO365

EVIDENCE FOR THE PRESENCE OF TWO GENETIC POPULATIONS OF THE BUSH-CRICKET *BARBITISTES VICETINUS* (GALVAGNI & FONTANA, 1993) (ORTHOPTERA, TETTIGONIIDAE) IN THE OUTBREAK AREA

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Barbitistes vicetinus is a recently detected bush-cricket, endemic of confined hilly areas in north-eastern Italy (Euganean and Berici Hills). It was considered rare until 2008, when repeated outbreaks occurred in ever-increasing surface areas. In this context, heavy damage to forests and neighbouring crops (mainly vineyards and olive groves) as well as annoyance to people living close to the attacked areas were recorded. Since outbreaks occurred only in the last decade, few data are currently available on ecology, biology and genetics of this species. The genetic population structure of *B. vicetinus* across its distribution areas was studied here through two mitochondrial markers, the protein-coding gene cytochrome c oxidase II (COII) and the non-coding control region (CR). More than 100 specimens coming from both the Euganean and Berici Hills were analysed. Results highlighted mitochondrial differentiation between the samples from the two distribution areas, assuming the presence of two distinct populations. This result was confirmed by the analysis of molecular variance (AMOVA) that revealed significant values of the fixation index (Fst). The analysis of the demographic history events suggested the presence of a past demographic expansion for both the Euganean and Beric populations. The time of separation between the two populations, estimated through the relation $\tau=2ut$, could coincide with last glaciation period when vegetation present between the two hilly areas could have experienced modifications becoming unsuitable for *B. vicetinus* and leading to the isolation of the populations. Besides confirming the endemic origin of *B. vicetinus*, this study provides interesting information concerning past demographic events of this outbreaking species.

Keywords: Outbreak, genetic variability, tettigoniids, mitochondrial DNA

PO366

SOIL HARDNESS AND SOIL TEXTURE OF NESTING SUBSTRATE IN SOLITARY AND SOCIAL DIGGER BEES AND WASPS (HYMENOPTERA: APOIDEA)

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Among the ecological factors that seem important in the evolution of sociality there are those associated with energetic efficiency: if a determined ecological factor exerts a strong pressure on the fitness, cooperative behaviours can evolve as a possible response to limit such pressure. Within Hymenoptera, the superfamily Apoidea includes bees and predatory wasps, many of them building their nest in the soil. Soil digging is energetically an expensive activity and its cost is proportional to the hardness and texture of the soil. A possible solution to limit the energetic cost associated with soils particularly difficult to dig could be collaborate in building nests through cooperative behaviour (sociality). If this hypothesis is true, we should find that species that belong to evolutionary lineages (genera) where sociality has evolved dig in harder and more compact soils than species that belong to solitary lineages. Using data available in literature and in unpublished studies on >60 bee and wasp species, we found that hardness and texture of nesting soils were highly variable, with such variation possibly and partially linked to the evolution of sociality. In particular, species from social lineages dig in soils significantly harder and richer in silt than species from solitary lineages. On the other hand, sand and clay components of soil did not differ between the two groups of species. The texture profiles of the analysed species, when represented in a classical soil triangle

classification, showed that most species fall in the spaces “sandy loam”, loamy sand” and “sand”, with some species, especially from social lineages, falling in the spaces “clay loam” and “silt loam”. New data on species from additional lineages and a formal correction for phylogeny are necessary to confirm the preliminary evidence that soil characteristics drove the evolution of sociality in ground-nesting Apoidea.

Keywords: Nest, soil hardness, soil texture, digging, sociality, Apoidea

PO367

RESEARCH ON MTDNA (COI) REGION OF *LOBESIA BOTRANA* (DENIS & SCHIFFERMÜLLER) (LEPIDOPTERA: TORTRICIDAE) IN COASTAL AND INLAND PARTS OF TURKEY

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Lobesia botrana (Denis & Schiffermüller) (Lepidoptera: Tortricidae) is a main pest for vineyards in Turkey. The one of the control tactics to suppress its population under the economic threshold level is biotechnical and biological control in the region. Both tactics need determination of the pest or biotype, accurately. So the aim of the study is determine differentiation of the *L. botrana* by molecular methods. *Lobesia botrana* larvae were collected and cultured to obtain adults from 43 vineyard areas at coastal (Adana, Mersin, and Antalya provinces) and inland parts (Kahramanmaraş, Adıyaman, and Gaziantep provinces) of Turkey. Mitochondrial DNA cytochrome oxidase (COI) gene was used for phylogenetic analysis, and USA and Australia reference populations from NCBI were compared with our samples. Totally 675 bp nucleotide sequences were obtained from 50 samples. After alignment of sequences by Mega 6.0, the haplotype number was detected by using DnaSP 5.5. Arlequin program was used for gene flow (Nm) and binary genetic distance (Fst) values. Seven haplotypes were detected in the sampled area. The coastal area had more haplotype and haplotype diversity (Hd) than inland. While Fst between coastal and inland was 0.63473 as the highest, between coastal and others (USA and Australia) were 0.21300 as the lowest. Nm was 0.28774 between coastal and inland, but 1.19579 was seen between coastal and others. Turkey Haplotypes, 2, 6, and 7, clustered the same branch at the phylogenetic tree than differently others. Overall, the results show Turkey *L. botrana* populations are genetically close to others country samples then inland population, but not too big differences with inland. This result may still be related to the use of local grape varieties and traditional production methods in the inland region. On the contrary, coastal region generally use the commercial grape varieties imported from other countries and close to transportation areas.

Keywords: Grapevine moth, cytochrome oxidase (COI) gene, population genetic, Turkey

PO368

INVESTIGATION OF GENETIC DIVERSITY IN MTDNA (COI-COII) REGION OF *CERATITIS CAPITATA* WIEDMANN (DIPTERA: TEPRITIDAE) IN THE EASTERN MEDITERRANEAN REGION OF TURKEY

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In this study, highland and lowland regions of Adana, Mersin and Hatay provinces of the Eastern Mediterranean Region of Turkey were surveyed to collect fruit infested with *Ceratitis capitata* Wiedmann (Diptera: Tephritidae). As a result, 999 *C. capitata* individuals were obtained from 31 different orchards and mitochondrial DNA sequences were obtained to assess genetic diversity. Isolated genomic DNA samples were amplified using PCR, and 914 bp nucleotide sequences of the COI-COII region were obtained via the C1-J-2792 (George) and TK-N-3772 (EVA) primer pair. Sequences from 103 individuals were obtained and aligned unambiguously using Mega6.0. Sequences were analyzed using DnaSP 5, which revealed the presence of seven haplotypes. Arlequin was used to estimate gene flow (Nm) and population genetic distances (Fst). Populations from Hatay and Mersin Provinces were the most differentiated (Fst=0.00486), while those from Hatay and Adana showed no differentiation (Fst=-0.01604). However, gene flow estimates showed that the populations in Adana and Mersin provinces were panmictic (Nm=∞), while gene flow was more limited between Hatay and Mersin (Nm=604.84). Haplotype diversity and genetic diversity of populations in the higher elevations was greater than the populations in lowland areas. Finally, phylogenetic analysis of the Turkish *C. capitata* individuals in the context of other samples worldwide (China, USA, Australia, Greece) shows that Turkey’s populations are differentiated from populations other countries, and that the highland populations are also uniquely differentiated.

Keywords: Mediterranean Frit fly, population genetics, highland populations, Turkey

PO369

PRELIMINARY RESULTS OF GENETIC DIVERSITY IN A GEOGRAPHICALLY BROAD SAMPLE OF *HELIOTHRIPS HAEMORRHOIDALIS* POPULATIONS

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Heliothrips haemorrhoidalis (Thysanoptera, Thripidae) is a worldwide distributed species, mainly through tropical and subtropical countries, which are harmful to protected crops (*i.e.* ornamentals) and citrus orchards. Recently its populations have shown “host shifts” to forest plants (Quercus, Pinus). Originally native to South America, its invasive populations show both thelytokous and bisexual reproduction. Moreover, the most recent literature data demonstrate that the sexual reproduction still occurs in the native rang and suggest the possible derivation of the global-conquering thelytoko us strains. Our study starts from these considerations and aims at search for genetic diversity inside the haemorrhoidalis natural populations in order to point out some basic biological aspects (*i.e.* reproduction) that support the host-selection and pest activity. A polymorphic molecular analysis has been carried out on three strains of the species collected in Brazil, Australia and South Italy, by means of a methodology that provides for the amplification and sequencing of a portion of the mitocondrial cytocrome oxydase I gene (COI). DNA was extracted from each thrips specimen using the protocol modify by Kawasaki and PCR was obtain ed using specific primers RON and C1N 2353. Each specimen wash omogenized whit the use of a mixer mill, then the tubes were incubated at 95°C for 20 minutes. The PCR products were cleaned over silica column (Quiagen) and a sequencing reaction with 20ng of DNA was perfomed in order to obtain DNA sequences, generated through an Applied Biosystems automated sequencer 3130xl. The COI sequences were edited by eye with Bioedit 7.2.5, virtually translated into the corresponding amino acid chain (to detect frame-shift mutations and stop codons), aligned, and used for a preliminary phylogenetic evaluation of genetic diversity and phylogenetic relationships between *H. haemorrhoidalis* populations.

Keywords: Heliothrips, host shifts, COI sequences, South America

PO370

DIVERSITY OF INTERNAL GENITALIA FUNCTIONING ACROSS A PHYLOGENETIC NESTED SET OF BUSHCRICKET SPECIES

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The males of some bushcricket species have spiny “titillators”. These genitalia are species specific and obviously subject to sexual selection. The titillator function is still not fully understood; hitherto it has only been investigated in detail in the Tettigoniinae bushcricket *Metrioptera roeselii*. There is strong evidence for titillators to act as copulatory courtship devices: they stimulate females by rhythmic copula movements and support the spermatophore transfer. Especially mechanical manipulation of titillators alters a female’s copulation behaviour. We studied the function of the titillators in four different species with a gradual phylogenetic relationship: three Tettigoniinae species with paired titillators (*M. roeselii*; *Pholidoptera littoralis*, *Tettigonia viridissima*) and one Phaneropterinae species with a single titillator (*Letana inflata*). For that purpose, the consequences of titillator manipulations were tested in mating experiments. Asymmetric manipulation (removing the spines of one titillator) in *M. roeselii* led to a resistance behaviour in 1/3 of the females. Similar results were found in *L. inflata*, where 2/3 of the females walked during matings with titillator manipulated males. This behaviour resulted in a significant number of failed ampullae transfer. Here, titillators function as stimulators to suppress female resistance behaviour. In opposition, we found no measurable effect on the females when manipulating the males in *Ph. littoralis* and *T. viridissima*, suggesting that stimulation is not the main titillator function. However, ablations of titillators in *Ph. littoralis* resulted in males slipping out of the mating position. The titillators of *Ph. littoralis* maintain the mating position by pulling both mating partners closer together. Bushcricket titillators have a species-specific functioning. This calls for an integrated explanation for the genital evolution. Neither the male-control hypothesis advocated by examples with traumatic genital functioning in other insects, nor the copulatory courtship function, clearly supported for *M. roeselii*, can explain the results when testing a range of bushcricket species.

Keywords: Orthoptera, Ensifera, Tettigoniidae, titillators, sexual selection, female choice, stimulatory devices, genital manipulations

Session 8.
Insect Control Biotechnology

Use of RNA Interference for Insect Control

PO371

ASSESSING THE SAFETY OF DSRNA TARGETING *LEPTINOTARSA DECEMLINEATA* TO THE PREDATOR *CHRYSOPERLA CARNEA*

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RNA interference can be used to induce silencing of target genes in arthropod pests. While double strand RNA (dsRNA) can be designed rather specifically for selected target pests, non-specific silencing effects, either of the homologue of the target gene or elsewhere in the genome of non-target organisms, can occur if a sufficient sequence similarity exists. Furthermore, unintended effects due to non-sequence dependent mechanisms, *e.g.* immunostimulation or saturation of the RNAi machinery, have been hypothesized as well. Off-target effects have been most commonly observed in taxonomically related species, however they are possible even in different insect orders. Since not all arthropod taxa show similar sensitivity to dsRNA, the assessment of the selectivity of specific dsRNA during the development of dsRNA based pesticides or resistant plants is a very important goal of the process. In order to assess the selectivity of a dsRNA targeting *Leptinotarsa decemlineata* Say, we conducted laboratory experiments using the larvae of the generalist predator *Chrysoperla carnea* Stephens as non-target species, for which very little genomic data exist. Second instars of the predator were fed with a sucrose solution containing dsRNA at a concentration that is about 200 times higher than the one which proved to be lethal for *L. decemlineata*. Different types of food supply were tested and in order to mimic natural conditions, a mixed diet constituted of sugar solution and arthropod eggs was used. No statistically significant differences were found either in terms of mortality or weight increase of specimen which were reared until adulthood.

Keywords: RNA interference, environmental risk assessment, non-target effects

PO372

RNAI-MEDIATED IMMUNE SUPPRESSION OF PEST INSECTS TO ENHANCE THE IMPACT OF THEIR NATURAL ANTAGONISTS

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The need to reduce the use of pesticides in agriculture has fostered intense research efforts on the identification of new bioinsecticides of natural origin and of appropriate delivery strategies. In particular, new bio-inspired tools and strategies of pest suppression, which mimic the negative effects exerted by natural antagonists on host insects, are currently being developed, using novel molecular tools of gene regulation, like RNAi. In this context, we have previously demonstrated that RNAi-mediated silencing of an immune gene (*102 Sl*), targeted by a polydnavirus associated with a parasitic wasp, generates an immunosuppressed phenotype in *Spodoptera littoralis* (Lepidoptera: Noctuidae) larvae and enhances the virulence of the entomopathogen *Bacillus thuringiensis*. This proof of concept paves the way for developing novel technologies of pest control, based on immunosuppression as a strategy for enhancing the impact of natural antagonists. Here we explored different delivery strategies of dsRNA targeting the expression of the immune gene *102 Sl*. *S. littoralis* larvae fed on transgenic tobacco plants, expressing *102 Sl* dsRNA, or on artificial diet on which was layered a suspension of sonicated heat-killed *Escherichia coli* expressing *102 Sl* dsRNA, showed marked transcriptional down-regulation of the target gene and impairment of the cellular immune response. The immunosuppression of *S. littoralis* was confirmed *in vivo* by the increase of susceptibility to the entomopathogen *B. thuringiensis*. These results indicate that efficient delivery of dsRNA molecules have the potential to enhance the impact of natural antagonists. and thus contribute to the development a new "bio-inspired" strategy of pest suppression.

Keywords: Plant protection, *Bacillus thuringiensis*, biological control.

PO373

SENSITIVE RESPONSE OF RNA INTERFERENCE IN LEPIDOPTERAN AND DIPTERAN CELL LINES

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RNA interference (RNAi) is a tool for functional study in insects and other species. However, the RNAi response in lepidopteran insects is variable. In order to perform a fast and large-scale screen of RNAi response in Lepidoptera, a fast RNAi response system was built up in seven lepidopteran cell lines and two dipteran cell lines. All the cell lines could have RNAi response as low as double-stranded RNA (dsRNA) is only 3 ng. Among the nice cell lines, Bm5, SF21, S2 and Sl2 cell lines have a more sensitive response to dsRNA and are concentration dependent. LC50 showed that these four cells line could only need as low as less than 2 ng of dsRNA to have a fast RNAi response. To test if the sensitive RNAi response is related to the factors in RNAi pathway and viruses related to RNAi response, real-time PCR was carried out. Ago1, Ago2, Ago3, Dcr2 and R2D2 in RNAi pathway were detected in Bm5, BmN4 and S2 cell lines. While viruses FHV and MLV were detected in all the candidate cell lines. The dipteran cell line showed higher expression in most factors in RNAi pathway and FHV, while lepidopteran cell lines showed higher expression in R2D2 and MLV. The data obtained above could explain the difference response of RNAi in the different insect cell lines.

Keywords: Cell lines, RNAi response, Lepidoptera, Diptera, dsRNA, virus

PO374

EXAMINATION OF THE POTENTIAL OF VIRUS-INDUCED GENE SILENCING AND VIRAL-LIKE PARTICLES FOR ENVIRONMENTALLY SAFE INSECT PEST CONTROL

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RNA interference (RNAi), or RNA-mediated gene silencing, has shown enormous potential to be incorporated in the arsenal of "next-generation" insect pest control agents. The most important obstacle that hinders RNAi-based applications however is the efficiency of delivery of the dsRNA trigger to the targeted insect pests. Our studies focus on the potential to harness RNA viruses as a potent delivery system of RNAi in insects. In first instance, we have developed a plasmid-based production system for recombinant Flock house virus (FHV) that is capable to trigger specific gene silencing and enhance mortality in infected Drosophila cell lines and adult flies. Since FHV has a broad host range, the use of recombinant FHV for virus-induced gene silencing (VIGS) can be extended to other groups of insects. To accomplish this purpose, a production system for recombinant FHV in lepidopteran cell lines has been initiated. On the other hand, the use of viral-like particles (VLPs) can be considered a safer approach since no virus replication will occur after their introduction in the environment. Cytoplasmic polyhedrosis virus (CPV) can be considered an interesting starting point since it is a natural carrier of linear dsRNA molecules and its structure is known in great detail by cryo-electron microscopy studies. Production of structural proteins of CPV in different combinations is currently being carried out using the Multibac baculovirus expression system and protocols are being developed for purification of VLPs with different protein composition. Our goal is to develop VLPs that can be efficiently taken up by (lepidopteran) insect cells and to explore the conditions for loading with dsRNA molecules.

Keywords: RNAi, pest control, virus-induced gene silencing, viral-like particles, FHV, CPV

Biopesticides, Genetically Modified Organisms and Maintenance of Ecosystem Services: Can They be Profitably Integrated?

PO375

POTENTIAL OF PLANT EXTRACTS AS CONTROL TOOL FOR THE SOUTH AMERICAN TOMATO PINWORM

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The implementation of environmental friendly methods for the management of key crop pests is strongly required both by public opinion and European regulation. Among sustainable control tools botanical insecticides, and in particular plant essential oils (EOs) have shown a good potential in controlling insect pests, as well as managing plant pathogens. Tomato has a very high social and economic relevance in Europe and the whole Mediterranean area. Several key insect pests (*e.g. Tuta absoluta*, aphids and whiteflies) affect the tomato industry in the Mediterranean. Their control often relies on agrochemicals which can disrupt the existing integrated pest management programs (IPM) and have unwanted side effects. In this context, laboratory trials were conducted to assess the insecticidal activity, through contact and ingestion exposure route of seven commercial essential oils (EOs), against *T. absoluta*. Toxicity to various instars and repellency towards adults, as well as the potential toxic effects on tomato plants, were evaluated. Chemical characterization of the tested EOs was carried out by GC-MS and GC/FID. Both the zeta potential (ζ) and the particle size distribution of the formulations developed were assessed. Fennel and anise nanoemulsions caused significant mortality of *T. absoluta* eggs. In the ingestion toxicity trials, all the tested EOs caused significant mortality. Fennel, artemisia, sage, lavender and peppermint EOs significantly reduced the number of laid eggs on sprayed plants. The formulations developed had nanometric scale dimensions (113-146 nm) and good stability over time (average ζ: -15.4 mV). Chemical characterization of the tested EOs highlighted some simpler [*e.g.* anise and clove EOs were mainly constituted of anethol (87%) and eugenol (91%), respectively] and some more complex phytochemical profiles. These results provide the scientific bases for further studies aimed at integrating these EOs into IPM packages in tomato.

Keywords: Essential oil, IPM, nanopesticide, *Tuta absoluta*, botanical

PO376

CIS-REGULATORY SEQUENCES SEARCHING FOR A Y-DRIVE SYSTEM IN *AN. GAMBIAE*

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Gene drive system is based on selfish genetic element that manage to cheat the natural law of evolution, being inherited more than 50% in the offspring. They are able to "drive" themselves and nearby genes through a specific population over many generations. Selfish elements harbored on the sex chromosomes are able to reduce fertility and distort the sex ratio of progeny, with different regions of the genome conflicting during meiotic transmission of the sex chromosomes. Regarding the reduction of mosquito population, this technology is expected to be extremely species-specific of malaria transmitting Anopheles and self-sustaining to spread on its own. For this purpose, we are focusing our attention on the production of a Y-

drive system for *An. gambiae* using the endonuclease I-PpoI. The problem related to the expression from the Y chromosome is to address to the MSUC, a phenomenon of early transcriptional repression and heterochromatization of the sex chromosomes that happen during the pachytene stage of meiosis, while the rest of the genome is actively transcribed. The expression of the endonuclease from the Y chromosome, using the spermatogenesis-specific β2 tubulin promoter, is silenced. In order to achieve the Y-linked meiotic expression, we have selected some cis-regulatory sequences of genes, which are active during the spermatogenesis, in order to test them on the possibility to evade the MSUC. We are testing the different promoters for the expression of the endonuclease I-PpoI fused with a fluorescent marker eGFP. We will present some preliminary results about the building of a Y-drive system in *Anopheles gambiae*.

Keywords: *An. gambiae*, Gene Drive, Y Chromosome, cis-regulatory sequences

PO377

CONSIDERATIONS FOR BIOSECURITY OF GENETICALLY MODIFED INSECTS WITH RESPECT TO THE NIGERIAN BIOSAFETY LAW

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Genetically Modified Organisms (GMOs) holds a huge promise for Nigeria, the most populous country in Africa. Genetically Modified Insects (GMIs) as GMOs hold great promise for Nigeria in Agricultural and public health concerns with implications for pest control and enhancement of productivity for agriculture. The Cartagena Protocol on Convention on Biodiversity has generated interest leading to legislation in different countries including Nigeria to regulate the practice and effect of products of modern biotechnology on biodiversity. The Nigerian Law is known as the National Biosafety law. With this law, it is expected that the floodgate for various GMOs (including GMIs) finding their way into Nigeria with their huge potentials for agriculture, environment, commerce, public health, animal health and scientific research. There are however considerations to the introduction of the GMIs requiring elaborate Environmental Risk Assessment (ERA). Also, public ignorance and opposition to GMIs, inadequate institutional capabilities and paucity of qualified technical manpower for monitoring and enforcement of the law also need to be considered. The Nigeria Biosafety Association has been involved in training and membership drive but there's no emphasis on GMIs and their containment for biosecurity considerations. Also, in Southwest Nigeria only one institution has capacity for biosafety testing but without focus for biosecurity. If these bottlenecks are addressed, GMIs holds promise for Nigeria to reap the dividends of sustainable development.

Keywords: Biosafety, Biosecurity, Genetically Modified Organisms, Genetically Modified Insects, Environmental Risk Assessment, Nigeria Biosafety Law, Nigeria Biosafety Association

PO378

CLONING, MOLECULAR CHARACTERIZATION AND TISSUE EXPRESSION OF AN OCTOPAMINE/TYRAMINE RECEPTOR FROM SPOTTED WING DROSOPHILA (*DROSOPHILA SUZUKII*)

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Spotted wing Drosophila (*Drosophila suzukii*) is a polyphagous pest arrived in Europe in 2009 able to infest a growing number of fruit and vine species, causing considerable economic damage. *D. suzukii* grows very rapidly (seven to fifteen generations per year) and shows a remarkable ability to adapt to climatic conditions and to new host plants. These characteristics make its populations particularly difficult to control. Octopamine (OA) and tyramine (TA) biogenic amines are present in traces in vertebrates, while in invertebrates they act as substitutes for adrenaline and noradrenaline. Indeed, these amines regulate numerous physiological processes in insects. They exert their effects by binding to specific receptor proteins that belong to the superfamily of G-protein coupled receptors (GPCRs). In this work, we have isolated complementary DNA (cDNA) coding for an amine receptor from *Drosophila suzukii* (DsTyr). The cloned cDNA is about 1.8kb long and encodes for a 601 amino acids protein. This polypeptide presents the classical seven transmembrane domains as revealed by hydropathic profile analysis. BLAST analysis of the sequence shows a high identity (>98%) to the octopamine/tyramine receptor from *Drosophila melanogaster*. DsTyr1 deduced sequence will be compared to the amino acid sequence of octopamine/tyramine receptors from other insects. Furthermore, the various receptor sequences will be characterized by phylogenetic analysis. The expression level of the receptor will be studied by qRT-PCR analysis in different parts of *D. suzukii* male and female body (head, thorax and abdomen). With this work, we present a first structural and functional description of an octopamine/tyramine receptor from *Drosophila suzukii*.

Keywords: *Drosophila suzukii*, octopamine, tyramine, biogenic amines, G-protein coupled receptor, qRT-PCR

Session 9.
Insects and Global Food Production

Feeding the World: How to Control Insect Pests of Crops Without Damaging the Planet

PO379

THE ELUCIDATION OF THE PHEROMONE PROFILE OF *DROSOPHILA SUZUKII* WITH GC-MS

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The development of state of the art integrated biological control methods are based on the use of semiochemicals, the organic compounds (hydrocarbons) that transmit chemical signals. These volatile pheromones are produced and used by insects for communication and identification. When the pheromone profile of a species in well known, it can be used for insect population management and control. *Drosophila suzukii*, *D. suzukii*, the fly Spotted Wing Drosophila (SWD), is one of the most important polyphagous pest of many small fruits, specifically stone fruits (berries, grapes, peach etc.). *D. suzukii* is a relatively new pest in the Americas, which has very recently appeared in in Italy (2011) and in Greece (2014) causing significant damage to crops. This is due to the fact that the ovipositor of the female can cause physical damage to the host fruit upon insertion, rending it soft and thus of no commercial value. Further damage is caused as the larvae are feeding on fruit flesh. Since the available data of *D. suzukii* pheromone profile is still limited, further research is required for the successful deployment of an Integrated Pest Management for this pest. In this work we will present the analytical data of the pheromone profile of both the male and female *D. suzukii*. These extracts from the fly cuticle,under different conditions, are analysed using a gas chromatograph coupled with a mass spectrometer. Compounds were separated and their structure elucidated based on their retention time and mass spectra profile. Several new compounds were detected and identified for both gender, which will hopefully allow for the development of IPM for *D. suzukii*.

Keywords: *D. suzukii*, Hydrocarbons, Pheromones, GC-MS

PO380

PHYTOHORMONES INDUCED ANTIXENOSIS IN SUSCEPTOIBLE VARIETY OF OKRA (*ABELMOSCHUS ESCULENTUS* L.) AGAINST WHITEFLY (*BEMISIA TABACI*)

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Whitefly is known to cause significant damage by sucking the cell sap and as a vector of many diseases. The present study was carried out to determine the feeding stress induced resistance in okra against whitefly. The experiments were conducted in the field in two growing season in 2017 (Feb-March and July-August). During the 1st season, susceptible variety was screened out by cultivating ten okra cultivars (China Red, Pen Beauty, OK-1304, OK-1315, Green wonder, Parbhani Karanti, Ikra, OK-1307, Kiran and Anarkali). The susceptible variety was subjected to the application of plant phytohormones (Benzoic Acid, Salicylic acid, Citric acid, Kh2PO4, K2HPO4) after 15 days of emergence to induce resistance and increase the plant vigor. Morphological parameters were also measured and collected the healthy and damaged leaves for biochemical analysis. After analysis results were indicated that OK-1304 and Pen Beauty were resistant against *Bemisia tabaci* infestation with a mean population 4.100/leaf and 4.977/leaf, respectively. The OK-1307 was proved to be highly susceptible with mean population 10.220/leaf. Population correlation analysis revealed negative correlation with humidity and positive correlation with temperature. Salicylic acid and citric acid was effectively reduced the pest infestation as 4.637 adult/leaf and 4.873 adult/leaf respectively at 0.5% concentration while maximum whitefly population was reported on control (10.017 adult/leaf). The biochemical analysis revealed that catalase concentration was decreased in infested leaves while POD, phenolic and SOD was increased. These compounds acts as resistant compounds against insect feeding and can be used to develop resistance at genotypic level against *Bemisia tabaci* by breeders.

Keywords: Whitefly, Antixenosis, Okra, Phytohormones

Insects and Global Food Production

PO381

WHAT DO PEOPLE WANT TO KNOW ABOUT INSECTS AND ENTOMOLOGY?

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Insects are important and fascinating. How do researchers and enthusiasts effectively communicate that message to a wider audience? How does that message compare with what the public usually wants to know about insects? Entomology provides us with charismatic examples for explaining a range of scientific concepts to the public, they are ecologically and economically crucial around the world and insects can be a popular topic, capable of attracting a high level of media attention. Their presence in almost every terrestrial habitat means that everyone is familiar with insects. This familiarity is informed by good or bad experiences, both of which can be used by scientists as a starting point to increase people's understanding of insect science and the work of entomologists. To support greater understanding and awareness, the Royal Entomological Society organises a full programme of outreach activities for all ages and abilities. The benefits and challenges of an intensive outreach program will be presented, with insights on how entomologists and insects are perceived by the public. Interesting techniques for engaging the public with entomology will be discussed, using specific Royal Entomological Society events and activities as examples.

Keywords: Outreach, public engagement, science communication, education

Entomophagy: Eating Insects as a Contribution to Global Food Production

PO382

ENTOMOPHAGY: EXPLORING THE NUTRITIONAL IMPORTANCE OF INSECTS IN AFRICA

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The practice of eating insects is a very old practice around the World and insects contribute about 40 percent animal protein required in rural villages and towns in Africa where majority of the people depend on animals from the wild which stock is fast declining as a result of over exploitation, environmental degradation, habitat fragmentation, diseases, pests and so on. Insects are abundant, cheap, readily available and sustainable in Africa because of the favorable environmental conditions of temperature and relative humidity that enhance their survival. Across the globe, Africa is one of the leading continents that consume a wide diversity of more than two thousand species from different orders. The insect groups consumed include termites, bees, beetles, wasps, cicadas crickets and so on. Edible insects could also be used to feed livestock and fish. Insects could be raised sustainably as they require very small space unlike livestock which is prided over its negative attribute as the highest user of agricultural land. Also it is fairly well established that insects do not deplete the ozone layer and consequently are not efficient polluters of the environment. In addition their feed to flesh conversion is very high and they could be harvested over a short period of time as the fecundity rate is very high. The forgoing portends that Entomophagy could be a way out of protein depletion challenges facing Africa and other less developed countries and thus could be a panacea for malnutrition plaguing many of the countries.

Keywords: Entomophagy Edible insects game malnutrition Africa sustainable livestock

PO383

REPRODUCTIVE PERFORMANCE OF *TENEBRIO MOLITOR* FED ON DIET CONTAINING YEAST

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The mealworm (*Tenebrio molitor*) is among insects one of the most interesting protein source for feed and food, but its rearing costs are an obstacle for mass production because are less competitive than typical protein supplies. In order to optimize the biomass production, much has been said about the factors affecting larval development (temperature, humidity, photoperiod, food quality, population density, oxygen concentration) but very less about the role of diets on performance of adult insects. The aim of this study was to assess the effect of brewer's yeast addition to a protein-rich diet.The trial has been performed into the ENEA Research Centre of Trisaia (MT), using a T. molitor colony reared since 2016. Four groups of 200 adults (C), just after they came out of the pupae, were fed on high protein (20.5%) commercial poultry feed and compared to four groups (Y) fed on the same diet enriched with ad libitum brewer's yeast. The rearing environmental chamber was maintained at a constant temperature of 28±2°C, 50±10% R.H. and 16:8 h (L:D) photoperiod. Water was supplied every 3 days by potato slices. The effect of yeast in the diet was assessed on adult survival, every 10 days, and on the number of larvae produced. The first results show that insects (Y) fed on the yeast enriched diet have a higher survival rate and produce more larvae than control group (C), although the basic diet used for both the theses already has B vitamins and high protein content.

Keywords: Alternative protein sources, adult mealworm, insect rearing, dietary supplements

PO384

COMPARISON OF THE MORPHOFUNCTIONAL PROPERTIES OF THE LARVAL MIDGUT OF *HERMETIA ILLUCENS* (DIPTERA: STRATIOMYIDAE) REARED ON DIFFERENT DIETS

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Hermetia illucens (Diptera: Stratiomyidae) is among the most promising organisms for the bioconversion of organic waste in proteins for feed production, because the larvae are able to grow on a wide variety of organic substrates and the dry-matter of the prepupae contains a very high percentage of protein with high nutritional value. One of the potential substrates for bioconversion by *H. illucens* is Fruit and Vegetable Waste (FVW), which could be provided in large amounts by large-scale retail trade and wholesale markets. However, in view of a possible application of this system, it is fundamental to evaluate the biological performance and the morphological, physiological, and molecular responses of *H. illucens* reared on this food substrate. In the present study we compared larvae reared on a standard diet for dipteran larvae and on FVW. In particular, after evaluating the growth performances, we focused our attention on the midgut, which is responsible for nutrient digestion and absorption. The morphological changes occurring in this organ after the ingestion of the two diets were investigated by optical microscopy. Moreover, an evaluation of midgut functionality was performed. An analysis based on enzymatic histochemistry was carried out to evaluate specific differences in lipids and glycogen accumulation in midgut cells. Finally, we assessed the activity and the mRNA expression levels of enzymes involved in digestion, focusing on proteolytic and amylolytic ones. Our results demonstrate that the extraordinary feeding plasticity of this insect corresponds to an extraordinary biological, morphological, physiological and molecular plasticity,that allows larvae to perform an effective bioconversion of FVW, opening up interesting application perspectives. This work was supported by Fondazione Cariplo (Insect bioconversion: from vegetable waste to protein production for fish feed, ID 2014-0550).

Keywords: Bioconversion, *Hermetia illucens*, Midgut.

PO385

REPLACEMENT OF *HERMETIA ILLUCENS* LARVAL MIDGUT DURING METAMORPHOSIS

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The Black Soldier Fly (BSF), *Hermetia illucens* (Diptera: Stratiomyidae), is studied for its bioconversion ability of organic waste. Moreover, the high nutritional value of prepupae and pupae makes this insect useful for the production of feedstuffs. Despite the great interest for this species, data on BSF biology remain scarce and basic biological studies on BSF are limited. In particular, no information on the morphology, physiology and development of the midgut, which is implicated in food digestion and nutrient absorption, is available in the literature. In the present study we performed a structural and functional characterization of the midgut during the larva-pupa and pupa-adult transition, to investigate how this organ changes during metamorphosis, a critical phase that, in holometabolous insects, leads to the rearrangement of different larval organs. To this aim, we analysed the morphology of the midgut epithelium, the behaviour of intestinal stem cells and the mobilization of long-term storage molecules, *i.e.*, glycogen and lipids. Moreover, we performed experiments to investigate the functionality of this organ during the remodelling process. Our results demonstrate that, similarly to other holometabolous insects, the larval midgut of *H. illucens* is completely removed during metamorphosis and a new pupal-adult epithelium is progressively formed by the proliferation and differentiation of stem cells. During this process, glycogen deposits and lipid droplets are depleted. Moreover, functional experiments suggest that the newly forming epithelium is endowed with digestive capabilities.

This work was supported by Fondazione Cariplo (Insect bioconversion: from vegetable waste to protein production for fish feed, ID 2014-0550).

PO386

MEAL PREPARATION, NUTRIENT CONTENT, AND MICROBIOLOGICAL ANALYSES OF *HERMETIA ILLUCENS* REARED ON VEGETAL SUBSTRATE

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The worldwide consumption of proteins for human and animal nutrition will dramatically increase in the next years because of the demand of a continuously growing human population. Moreover, climatic changes and food-feed-fuel competition sharpen this challenge to obtain more resources. In this perspective, insects could be a potential source of animal proteins and the black soldier fly (BSF), *Hermetia illucens* L. (Diptera: Stratiomyidae) is very interesting to this aim. According to the activities of the research project “INBIOPROFEED” a completely vegetal substrate has been explored to grow BSF larvae and to obtain feed material for aquaculture. In particular, *H. illucens* was reared on a vegetal substrate constituted by a mixture of fruits and vegetables representing daily wastes of a fruit and vegetable market of Northern Italy in the winter period. Larvae in the final instar were frozen and then dried at a temperature decreasing from 90°C to 60°C for the time necessary to reach a steady weight; the recorded humidity loss was of 67% with respect to the total fresh weight. Different methods were employed to extract oil and to obtain meal. The most efficient procedure was represented by treating the insects in an auger screw with pre-heating, which resulted in 63.8% fat extraction. Nutritional analyses were performed on the derived meal and oil, including the meal amino acid profile and the fatty acid composition of oil. Microbiological analyses showed the substantial absence of pathogens in significant quantity. The overall results demonstrate that rearing BSF by re-using commercial vegetable remaining of markets can represent an alternative to recycle this waste and to obtain a good feed material in term of nutritional and microbiological properties.

This work was supported by Fondazione Cariplo (Insect bioconversion: from vegetable waste to protein production for fish feed, ID 2014-0550).

Keywords: *Hermetia illucens*, vegetal substrate, compositional analyses, microbiological profile

PO387

EVALUATION OF MICROBIOLOGICAL AND CHEMICAL RISK OF *TENEBRIO MOLITOR* (COLEOPTERA: TENEBRIONIDAE) LARVAE INTENDED FOR HUMAN DIET

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Actually, in Europe there is an arising interest toward insects as alternative food for human diet and, in this trend, specific legislation is necessary to ensure quality and safety of insect-based food and to promote their acceptance by western people community. Edible insects as well as other farmed animals, might accumulate/carry biological and chemical hazards via feeding, breeding or industrial food processing chains and so act as vectors of microbiological and chemical hazards for human health. In consideration of the absence, in Europe, of well -defined regulations for insects production for human diet and to contribute in providing data on the risk profile in consuming *Tenebrio molitor*, a species included in the list of edible insects, the content of microbes, allergens and toxins were investigated in larvae reared in laboratory conditions. In particular, the analyses were performed to value the possible presence of some groups of microorganisms commonly used as a measure of hygienic conditions or quality of food and food products by EU legislation as bacteria, mould and yeast, toxins of fungal origin and allergens. Data were compared to EU threshold levels admitted in common animal foods and their derivatives intended for human diet.

Keywords: *Tenebrio molitor*, edible insects, bacteria, toxins, allergens

PO388

QUALITATIVE PARAMETERS OF DRIED EARTHWORMS (LUMBRICINA) FOR HUMAN CONSUMPTION

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Due to the increase in the number of people on earth raising the need for food. The biggest problem is human providing with protein-rich foods if they will continue to be obtained from traditional animal protein sources such as cattle, pigs, chickens, which breeding is closely related to the high consumption of resources and the adverse effects on nature. For the expected lack of food, especial, lack of protein rich food products, it need to search solutions. In Latvia is widespread rearing of the earthworms (Lumbricina) for the humus creating, but other possibility can be usage of earthworms in food. In order to be able to be used in human consumption, earthworms can be dried, but the chosen drying method can significantly influence product’s qualitative parameters, which are important in the preparation of food products. In the study used earthworms species was Eisenia fetida also known as the red wiggler. Before drying the earthworms were discharged of compost, after then kept in freshly chopped apples twenty-four hours, and finally they were blanched. In this study, heating method (+85±1 degrees Celsius in convection oven) and freeze drying was used for drying of samples. Earthworms were quickly frozen in -35±1 degrees Celsius before drying with a sublimation method. Dried earthworms were ground in powder. During research was determined different qualitative parameters and drying method influence on them was determined. Moisture content of samples using both methods was 5±0.1%, the same significant differences was not found in water activity (for both was in range 0.259-0.263). The pH of the lyophilized sample was 7.2, while for the heated sample it was 7.5. Using color in CIE L*a*b* system was found significant differences in color of both samples ($p<0.05$). The microbiological parameters do not exceed the acceptable level for dried dehydrated products for the samples tested. The results shows both methods are suitable and product obtained can be used for food and food products preparation. Current research has been supported by the European Regional Development Fund under the activity "Post-doctoral Research Aid", project No 1.1.1.2/VIAA/1/16/190 "New sources of protein for food in Latvia"

Keywords: Alternative protein sources, entomophagy, dried worms as food, earthworms processing

PO389

USING *HERMETIA ILLUCENS* TO BIOCONVERT THE WASTE FROM INSECT FARMING

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The European pet food market is the second largest in the world with an estimated value of US$21.5 billion. Approximately 8.59 million reptile and amphibian pets are kept across Europe and a thriving and well established industry rears the live feeder insects for insectivorous species. Two popular groups of feeder insects are locusts and crickets, and substantial quantities of organic waste are generated in their production consisting of frass, exuviae and uneaten food. Here we investigated the use of a saprophagous fly species, *Hermetia illucens* (Diptera: Stratiomyidae), the black soldier fly (BSF), as a bioaccumulator to recapture the nutrients in this waste with potential applications in the pet food, animal feed, or biofuel industries. BSF larvae were reared on four experimental diets consisting of the waste from either cricket farming, or locust farming. These were used either unmodified or finely chopped, and the Gainsville diet was used as a control giving five conditions. Trial substrates were given to young handling larvae ad libitum and maintained in a climate chamber. The suitability of these diets for rearing BSF was demonstrated by a low mortality rate, not significantly affected by diet, and the successful emergence of adults from all conditions. BSF larvae reared on cricket waste reached a significantly higher weight than those reared on the locust and control diets. Diet did not significantly affect time taken to reach the prepupal stage, except in chopped locust condition which took more days. We conclude that the waste from farming locusts and crickets represents a suitable substrate for rearing *H. illucens*, potentially establish a circular nutrient economy in the rearing of insects for the live pet food market.

Keywords: Black soldier fly, waste, bioconversion

PO390

QUALITY ACCORDING TO HARVESTING TIME AND PROCESSING METHOD OF *LOCUSTA MIGRATORIA*

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Locusta migratoria is an insect belonging to Acrididae and is the largest species of Acrididae insect in Korea. It is about 4.5 ~ 6cm in size. It contains more than 70% of high protein and nutrients, and is widely available for food and feed. It is widely used as a grasshopper in the world, and it is likely to be a food source in Korea. This study measured the change of quality according to harvesting time and processing method of the *Locusta migratoria*. The harvesting time is 5 days until 5 ~ 30 days after adult. After collection, male and female were separated and dried, and their nutritional and functional components were examined. The processing methods were drying, drying after blanching, roasting and frying. Nutrients, functional ingredients, physical properties and storage stability were investigated.

Keywords: *Locusta migratoria*, harvesting time, processing method

PO391

EFFECT OF SPENT MUSHROOM SUBSTRATE OF *PLEUROTUS ERYNGII* ON THE DEVELOPMENT OF WHITE-SPOTTED FLOWER CHAFER (*PROTAETIA BREVITARSIS*)

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The white-spotted flower chafer, *Protaetia brevitarsis* (Coleoptera: Cetoniidae), has been registered as a kind of human food in Korea in December, 2016. The larva has been known to have an important source for medicinal properties such as anti-cancer activity and prevention of liver-related diseases. However, the consumer market is depressed because of high price of the larvae produced. To promote consumption on the larvae, the production expense should be reduced. This study was carried out to evaluate spent mushroom substrate as diet source of *P. brevitarsis* larvae to improve the productivity and to reduce production costs of the larvae. The larvae were fed with spent king oyster mushroom (*Pleurotus eryngii*) substrate (P-SMS) with a control of fermented oak sawdust (FOS). The effect of the diets on the development of *P. brevitarsis*, contents of minor nutrient components in the larvae produced were examined. In P-SMS diet, the larval rearing period was shortest as 16.2 days, the increasing rate of larval weight and the cocoon weight were highest as 156.3% and 4.1 g, respectively. Pupation rate was 100% and the adult emergence was highest as 93.3% in P-SMS diet, with no difference with control diet. Total nitrogen content reared in the P-SMS diet was highest as 10.28%, and the minor component, particularly Fe, was higher as 145.8 mg/kg than other diets. The oviposition preference of adults showed no significant difference among the diets. It was estimated that P-SMS has about 667,960 Won in economic gains per 100 kg larvae compared to FOS.

Keywords: *Protaetia brevitarsis*, Spent mushroom substrate, *Pleurotus eryngii*, Diet

PO392

INFLUENCE OF DIFFERENT DIETS ON GROWTH AND DEVELOPMENT OF *TENEBRIO MOLITOR* (L.)

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Insects hold potential as a sustainable source of animal protein. In recent years a new and rapidly growing industry of insect-based foods has emerged. The yellow mealworm (YM), *Tenebrio molitor* (L.) (Coleoptera:Tenebrionidae), is one of the most important insect species being produced commercially for feed and food. The purpose of this study was to test the influence of different diets, including organic by-products and waste, on the larval growth performance. Newly eclosed first instars (n=50) were placed in Petri dishes (n=3) for each diet and maintained at 28±1°C, 60±5% RH, and 0:24h (L:D) photoperiod. The tested diets were: wheat flour (S1), wheatmeal (S2), wheatmeal:olive-pomace (3:1) (S3), wheatmeal:olive-pomace (1:1) (S4), wheatmeal:olive-pomace 1:3 (S5). Last larval instar fresh weight was significantly lower on S5 (0.07g±0.003g) with the best performance on S3 (0.13g±0.004g). Pupal weight was significantly lower in S5 (0.15g±0.004g) with the best performance on S3 (0.19g±0.004g). The larval instar number (through the analysis of the frequency distribution of head capsule width) ranged from 8 (on S1) to 20 (on S5). Different diets produced different larval development time and survival rates. The larval development time ranged from 3 months (on S2) to 5 months (on S5). The larval survival rate ranged from 38% (on S5) to 88% (on S2). The gross composition of the different diets is also presented. The results of this study highlight the importance of feeding substrates on larval growth performance of YM.
This study was financially supported by the Università Politecnica delle Marche, Ancona, Italy, within the project "Edible insects: new frontiers in food-FOODIN".

PO393

BIOASSAYS FOR THE OPTIMIZATION OF *HERMETIA ILLUCENS* (L.) EGG LAYING IN A PROTOTYPE OF INDUSTRIAL PLANT

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The black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae) constitutes a particular interest for the feed and food industry as its larvae have the capacity to convert large quantities of organic waste or byproducts into a protein biomass rich in fats, quickly and efficiently. Although the information on larvae development is abundant, the required knowledge of adult biology to produce large amounts of eggs remains a great challenge. This work represents a part of a regional project (ValoriBio, Emilia Romagna, Italy) wherein one of the main outputs is the construction of a pilot plant for *H. illucens* mass rearing, which includes a module dedicated to adult rearing. Therefore, a specific custom-made climatic chamber was developed, consisting of a thermally insulated container (1m^3 volume), with a transparent window bearing a light-emitting diode (LED) lights source (UV, green and blue LEDs) on top. Temperature and humidity were controlled through a Peltier cooler/heater system, an ultrasound humidifier and several humidity-temperature digital sensors. An exterior electronic board connected to a touch screen was developed to control and record the rearing parameters. The biological parameters used to establish the functionality of the prototype were: the patterns of oviposition, the egg weight and adult longevity. Bioassays were conducted at 27°C±0,5°C and 70±5% relative humidity, in three types of climatic chambers considering the use of different light sources (fluorescent tubes, white LEDs and the UV-green-blue combination of LEDs) and the flies' diet (water and sugar, just water or nothing) as treatments. The results allowed the identification of the optimal conditions to obtain the highest amounts of eggs in the shortest time.

Keywords: Black soldier fly, reproduction optimization, insect mass rearing, automatized pilot plant, light sources

PO394

POPULATION DENSITY AND OVIPOSITION SITE SELECTION OF BLACK SOLDIER FLY, *HERMETIA ILLUCENS* (DIPTERA: STRATIOMYIDAE) FOR EFFICIENT PRODUCTION

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The black soldier fly is economically important because its prepupae are used as feed for many animals, including fish and swine. Such efforts require an understanding of optimal mating and oviposition techniques. Specifically, adult densities and cage size may both improve the efficiency of mass egg production. Our study used four sizes of nylon cages (1.0×1.0×2.5 m, 1.5×1.5×2.5 m, 2.0×2.0×2.5 m, 2.5×2.5×2.5 m) and three density treatments (4 kg, 8 kg, and 10 kg of pupae) to investigate optimal habitat size and adult density. We found that cage size (independent of density) did not significantly influence female fecundity (number of egg clutches and egg weight), whereas higher densities increased egg number and weight regardless of cage size. The ability of organisms to select suitable habitats for their survival and reproduction can have profound influences on their immediate Darwinian fitness and their potential for further evolution. Habitat selection and more specifically oviposition site preference (OSP) behaviors appear to have played an important role in the evolutionary divergence. The objective of this study was to investigate OSP by black soldier fly. We asked two questions: How sunlight stimulates the adult to oviposit and what substrates stimulate the oviposition of adult.

Keywords: Black soldier fly, oviposition

PO395

USE OF THE BLACK SOLDIER FLY AS A BIOCONVERSION TOOL AT THE INDUSTRIAL SCALE

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The larval stage of the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), are extremely voracious and able to consume a wide range of organic materials, ranging from fruits and vegetables to animal remains and manure. This ethological characteristic is particularly interesting for waste management at an industrial scale. The extraordinary ability to accumulate high levels of proteins and lipids, allows the use of resulting larvae as animal feed. Vegetables and fruit by-products are promising rearing substrates for insects produced for this purpose. In order to examine the effect of different diets on insect growth, lipids and larval proteins content and to evaluate pH values changes in diets used, approximately 10,000 larvae/unit were reared on six substrates: 1) apple, 2) banana, 3) spent grain from brewery waste, 4) apple and banana, 5) apple and spent grain, 6) banana and spent grain. High growth rate was observed in all the tested diets with differences among larvae final weights. Larvae fed with the apples and spent grain mix reached the highest final weight (1653 g±354.9366 g), the highest percentage of crude proteins (48.01%±3.7%) and showed the highest amount of diet bioconversion, while larvae fed with spent grain showed the highest growth rate, reaching their maximum weight in only six days (1.75 g±0.22 g/10 larvae). Although the initial pH value was dependent on the six different substrates, all tested diets reached a basic pH value at the end of the trial. This study shows that waste management through black soldier fly bioconversion processes represents a new economically important resource and opens new perspective of a sustainable environmental friendly industrial development. Moreover, the study demonstrates that the choice of the substrate has a direct influence on larval macronutrient composition and therefore on the biological market value.

Keywords: *Hermetia illucens*, waste management, bioconversion, valuable products

Session 10.
Medical and Veterinary Entomology

Epidemiology and Management of Insect Vectors

PO396

AEDES AEGYPTI INCREASE SURVIVAL RATES (DIPTERA: CULICIDAE) AS FUNCTION OF TEMPERATURE INCREASE

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Considering that vector insects such as *Aedes aegypti* adapts well to thermal differences, the present study aimed to evaluate the effect of a gradual temperature increase on the survival rates of these mosquitoes from different bioclimatic conditions. Generations F1, F2 and F3 of the insect, captured in the cities of Campina Grande, Agreste region, and Patos, in the backwoods region, both located in the Paraiba state of northeastern Brazil, were reared in the laboratory and subjected to 26 to 32°C, gradually increased by 2°C until the end of F3. There were significant interaction between mosquitoe's survival and temperature (F1.16=364.49; p<0.001), with a reduction in the survival rates due to temperature increase for the populations of C. Grande (r2=0.844; p<0.001) and Patos (r2=0.625; p<0.001). However, there was an increase in survival rates between F1 and F3 generations subjected to the same temperature (F1,6=26.107; p<0.001) for both populations, indicating possible acclimatization of these insects. The maximum temperature (32°C) allowed the development of the population of Patos for three generations, suggesting that this occurred due to the climatic characteristics of population's origin region and its biotype. Our results show that although the increase in temperature decreases the insect's survival, surviving generation tends to be able to survive and thrive in higher temperatures. This strengthens the adaptability theory of these mosquitoes, a concern with future projections for temperature elevation, especially for sites with higher demographic densities and cases of dengue and other diseases caused by A. aegypti.

Keywords: Acclimatization, Vector, Mosquitoes, Semiarid, Dengue.

PO397

A CHECKLIST OF SCORPIONS IN IRAN (BY 2017)

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Background and Objectives: In the last 50 years, scorpions have been studied from different biological and toxicological aspects in Iran. These efforts have been more focused on identifying different species of this animal. The present study aimed at compiling an updated checklist of scorpion species in 2017. *Materials and Methods:* Scorpion, species, classification, Iran, and family were taken as keywords and were searched in the websites related to credible academic journals and scientific databases such as Web of Science, Ovid, PubMed, Systematic Review, SID, Iran Medex, Scirus, Google Scholar and Medline. The search was more focused on species identification and included all articles published by 2017. A total of 150 articles were examined, out of which 75 were selected as they involved the scorpion species found in Iran. *Results:* From among the 64 scorpion species; in 20 genera; reported in Iran, 86% belonged to the Buthidae family, 9.5% to the Hemiscorpiidae family, and 4.5% to the Scorpionidae family. The species were mainly reported to inhabit southern and southwestern Iran. The number of scorpion species reported in Iran has increased in the past 50 years. This trend will continue as more accurate studies are going to be conducted in this field. Many Buthidae species are venomous, with less than 20 being fatal to humans. The Scorpionidae species identified in Iran include *Scorpio maurus* and *Nebo hengamicus*. The *Hemiscorpius lepturus* species is one of the dangerous members of the Hemiscorpiidae family. In Iran, one genus and 6 species of the Hemiscorpiidae family have been observed and reported. *Conclusions:* Southern and southwestern provinces will be the richest in species diversity. However, the species diversity decreases as we move away from the southwest towards the northeast and the northwest.

Keywords: Checklist, Scorpion, Species, Scorpionida, Iran

PO398

OPPORTUNISTIC BLOOD-FEEDING BEHAVIOUR OF UK CULICOIDES AND IMPLICATIONS FOR DISEASE SPREAD IN ZOO ANIMALS

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Some species of Culicoides biting midges are capable of transmitting bluetongue virus (BTV), Schmallenberg virus (SBV) and African Horse Sickness virus (AHSV) in northern Europe. Zoos are home to a wide range of 'at risk' exotic and native species of animals. These animals have a high value both in monetary terms and in terms of their conservation significance and breeding potential. Therefore, it is necessary to understand the risk of these viruses to zoo animals by characterising the Culicoides fauna at zoos and determining which potential vector species are feeding on which zoo animals). Using light suction traps at two UK zoos, a total of 9.684 individual Culicoides were caught comprising a total of 20 different species. The six putative vectors of BTV, SBV and AHSV in northern Europe were found at both zoos and made up the majority of the total catch. Thirty five blood-fed midges were processed for blood-meal analysis. Vector species of Culicoides were found to be biting a wide range of birds and animals, including camels, elephants, rhinos and rhea. This shows that vector Culicoides are opportunistic feeders with little or no host preference. This behaviour confirms their ideal suitability for spreading diseases to a wide range of susceptible animals. Due to the susceptibility of some zoo animals to Culicoides-borne arboviruses, this study demonstrates that preventative measures would need to be taken in the event of a UK outbreak of one of these viruses. These findings are of great importance for the protection of zoo animals, which are highly valuable both in financial terms and in terms of their significant role in international breeding programmes for species conservation.

Keywords: Culicoides, blood feeding, zoo, bluetongue, Schmallenberg

PO399

PRELIMINARY DATA FROM THE MONITORING OF *AEDES ALBOPICTUS* (SKUSE, 1897) (DIPTERA: CULICIDAE) IN MEDIO CAMPIDANO PROVINCE (SOUTHERN SARDINIA), ITALY

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The occurrence of *Aedes albopictus* (Skuse, 1897) in Sardinia is known since 1994-95 (Mantega *et al.*, 1996; Contini, 2006; Cristo *et al.*, 2006; Marchi *et al.*, 2006; Culurgioni *et al.*, 2010). The only Sardinian Province with no records about *Ae. albopictus* was Medio Campidano, nowadays part of the Southern Sardinia Province, newly established in 2016. The monitoring activity was aimed to collect data about the presence and the seasonal abundance of *Ae. albopictus* in this territory, in order to plan the control of the species by larvicides based on Diflubenzuron. The monitoring of *Ae. albopictus* was carried out from May and October 2015, in 8 Municipalities namely: Gonnosfanadiga, Guspini, San Gavino Monreale, Sanluri, Samassi, Sardara, Serramanna and Serrenti. In the present study 40 ovitraps were set in 5 selected stations in each Municipality. The presence of the species was investigated also by the occasional setting of BG-Sentinel adult mosquito traps baited with BG-Lure (Biogents, Germany). Mosquito specimens were identified according to morphological keys (Schaffner *et al.*, 2001; Severini *et al.*, 2009). The monitoring revealed the occurrence of *Ae. albopictus* in all the investigated Municipalities, with the higher number of eggs in Sardara (2361 eggs) and the lower in Serrenti (263). BG-Sentinel trap collected specimens in all the eight localities. The higher and the lower number of adult mosquitoes collected (74 in Sardara and 11 in Serrenti), reflected the same results from the ovitraps. This contribution to the knowledge of *Ae. albopictus* in the Sardinian territory closes the data gaps about the species in this part of the island, and could be an important base for future control activities, mainly at the light of the concern for the recent Chikungunya outbreaks occurred in Anzio and in Rome in the last Autumn (Venturi *et al.*, 2017).

Keywords: *Aedes albopictus*, *Stegomyia albopicta*, Medio Campidano Province, Sardinia

PO400

MOSQUITOES SPECIES COLLECTED FOLLOWING WEST NILE DISEASE HUMAN CASES IN SARDINIA (ITALY), 2017

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West Nile Virus (WNV) is a mosquito-borne virus belonging to the family Flaviviridae. WNV is maintained in nature and amplified with a enzootic cycle between birds and mosquitoes, while humans and other mammals represent incidental or dead-end hosts. *Culex pipiens* and *Culex modestus* are considered the main vector species in Europe. West Nile Disease appeared for the first time in Sardinia during 2011 when an outbreak in horses and 4 human cases were reported. In the summer 2017, other 4 human cases were notified in the municipalities of Zeddiani, Solarussa, Palmas Arborea and Marrubiu (Oristano Province – Western Sardinia). Following these clinical cases an entomological monitoring was conducted outdoor of patient's houses. Mosquitoes were trapped twice in each site using a CDC Light trap, a BG Sentinel Trap and a Gravid Mosquito Trap. Mosquitoes were morphologically identified, sexed and then pooled in maximum 25 specimens according to species, location, trap and sampling date. All mosquito pools were tested for the detection of WNV by using Real Time RT-PCR. During the survey a total of 337 mosquitoes were captured. The species identified were: *C. pipiens* (239 specimens), *Aedes caspius* (54), *Ae. albopictus* (13), *C. theileri* (10), *Anopheles labranchiae* (8), *Ae. vexans* (6), *Ae. detritus* (2) and *Culiseta longiareolata* (2). Three damaged mosquitoes were identified only at genus level as Culex. A total of 46 mosquito pools were analyzed, and a single pool consisting by 4 not engorged females of *Ae. caspius* was WNV positive. *Aedes caspius* is considered a mammophilic and anthropophilic species and our findings would suggest that it is a potentially important bridge vector species for WNV

Keywords: West Nile Disease, Mosquitoes, Sardinia, Italy

PO401

EVALUATION OF ESSENTIAL OILS ACTIVITY ON *AEDES ALBOPICTUS* (DIPTERA, CULICIDAE)

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Aedes albopictus Skuse, the "Asian tiger mosquito", is currently retained as one of the most invasive species in the world and a public health threat as vector of viruses like Chikungunya, dengue and West Nile viruses. Recently, *A. albopictus* has been established as one of the vectors of Zika virus. The personal protection from mosquito bites remains a crucial tool for preventing many virus infections. Essential oils, complex mixtures of volatile organic compounds, derived from a variety of plant species, have shown considerable efficacy as repellents against mosquitoes. Furthermore, they are regarded as safer than conventional synthetic chemicals repellents. *A. albopictus* female responses to seven essential oils, extracted from *Pelargonium graveolens*, *Cymbopogon nardus*, *Lavandula officinalis*, *Melaleuca leucadendron var. cajaputi*, *Boswellia sacra* and *Boswellia carteri* tested at a range of concentrations, were evaluated using electroantennographic (EAG) analyses and olfactometric bioassays. Essential oils were analysed by Gas chromatography–mass spectrometry (GC-MS) system. EAG analysis, carried out by exposing *A. albopictus* females antennae to ascending concentrations of the oils, proved that the oil volatile compounds elicited antennal responses, thus demonstrating their capability to stimulate the peripheral olfactory receptors on the antennae. The dose-response curves revealed that the amplitude of the responses was dependent on the tested oil and the concentration. Olfactometric bioassays showed a repellent activity depending on the tested oil and the concentration and provided evidences of a good repellent activity against adult females of *A. albopictus*. GC-MS analyses revealed high contents of monoterpenes in essential oils tested.
Research supported by BIOECOLOGY S.R.L. Cavriago (Reggio Emilia), Italy.

Keywords: Asian tiger mosquito, volatile compounds, electroantennographic (EAG) analyses, olfactometric bioassays, Gas chromatography–mass spectrometry (GC-MS) system

PO402

INCIDENCE AND EPIDEMIOLOGICAL PROFILE OF SCORPION STINGS IN NORTHERN KHUZESTAN PROVINCE, SOUTHWESTERN IRAN: A DESCRIPTIVE – ANALYTICAL STUDY

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Background and Objectives: Scorpion sting is an important health challenge in the tropical and subtropical areas. It is a significant medical emergency, particularly in children. Scorpionism is considerable health hazard in Iran, especially in the rural area of south and south–west of Iran. Because of the necessity of knowing some features in such patients, the aim of this study was an epidemiological study of scorpion sting in Dezful, Shush and Gotvand Counties, located in the southwestern part of Iran. *Materials and Methods:* This research was a descriptive-analytical study. The information was gathered and recorded in questionnaires. Data were collected from 1756 scorpion sting cases, during 2013. Data analyzed by descriptive statistical methods using SPSS version 18. *Results:* The age distribution of cases showed that the largest rate of scorpion stings occurred among the 15-24 (22.4%) year old group. The most frequent of scorpion stung people were female (51.3%). The most frequent scorpion stings (18.2%) were happened at June. The highest incidence of scorpion sting (59%) cases in above mentioned counties during 2013 were taken place in villages. Legs were more at risk of sting by scorpions (39.7%). Most of scorpion sting (41.5%) cases occurred in spring. A statistical significant correlation was observed between scorpion sting and age, month, season and sting site on the body ($P<0.05$). Statistical analysis showed a non- significant difference between history of sting, gender and residence place. *Conclusions:* The results of this study indicated that most of the cases happened in June, spring, rural areas and legs.

Keywords: Epidemiology, Scorpion Sting, Iran.

PO403

THE EFFECT OF ELECTRON BEAM ON *CULEX PIPIENS* AND *AEDES ALBOPICTUS*

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The effect of the ionization energy on the two mosquitoes (*Culex pipiens* and *Aedes albopictus*) pupa was investigated as the hatching rate and longevity. The longevity of adult emerging in the pupa decreased depending on the exposure dose of Gray (Gy) in both mosquitoes. The hatching rate of *Cx. pipiens* treated up to 70 Gy did not show any difference with the control, but the eggs did not hatch 100% at 120 Gy exposure. These results showed that *Cx. pipiens* was sterilized at 120 Gy. However, *Ae. albopictus* showed a significant difference in hatching rate depending on the dose of Gy. The *Ae albopictus* become infertile at 70 Gy. The susceptibility of various insecticides to electron beam-treated mosquito pupae and control was evaluated in both mosquitoes. The effects of electron beam on mosquito adults were also investigated. Therefore, this study was to observe the effect of electron beam on mosquitoes and to investigate the possibility of sterile insect technique (SIT).

Keywords: *Culex pipiens*, *Aedes albopictus*, Electron beam, Sterile insect technique

PO404

CULICOIDES SPECIES (DIPTERA: CERATOPOGONIDAE) INVOLVED DURING BTV OUTBREAKS IN SARDINIA, 2017

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Bluetongue (BTV) is an infectious disease of ruminants transmitted by Culicoides biting midges. Nowadays, several species as *C. imicola*, *C. pulicaris*, *C. punctatus*, *C. newsteadi* s.l,. *C. scoticus* and *C. obsoletus* are considered BTV-competent vectors. In 2017, an epidemic of BTV serotype 4 has been reported in Sardinia, Italy. To investigate the role of Culicoides species involved in the BTV-4 transmission, an entomological survey has been carried out in some farms where clinical and virological findings have been confirmed. By using an Onderstepoort Black-Light Trap, Culicoides collections have been performed between August and September. Culicoides specimens have been morphologically identified to the species level, pooled, according to species, location and date, in maximum 25 parous females (females that have completed at least a gonotrophic cycle) and then assayed for BTV by Real Time RT-PCR. More than 173,000 Culicoides adults have been collected. *Culicoides imicola* has been the most abundant species captured (97%), followed by *C. newsteadi* s.l. (2%). The other species have been collected with very low numbers. A total of 77 pools have been analyzed and 68 were BTV positive, including *C. imicola* (52 positive /53 tested pools), *C. newsteadi* s.l. (9/12), *C. paolae* (4/6), *C. circumscriptus* (2/3) and *Obsoletus complex* (1/2). The fundamental role of *C. imicola*, *C. newsteadi* and *Obsoletus complex* in the BTV transmission has been confirmed. Besides this is the first time that BTV-4 was detected in both *C. paolae* and *C. circumscriptus*. Our results suggest that during an outbreak more Culicoides species could be involved in BTV transmission, probably related to the high level of viral circulation, and they could play a role, even if marginal, in the spread of the disease

Keywords: Bluetongue, Culicoides, Vector, Sardinia, Italy

PO405

Poster withdrawn.

PO406

TRAP DESIGN METHODOLOGY FOR VECTOR CONTROL

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Vectores de enfermedades como dengue, zika y chincungunya, viven en nuestro ambiente urbano y rural, allí desarrollan su ciclo de vida en contenedores artificiales o naturales. Afectan a 146 países y con picaduras inoculan enfermedades al hombre y otros animales. Los controles para evitar el contagio, como saneamiento ambiental, control químico o control biológico no son suficientes, es necesario desarrollar trampas para monitoreo, captura y eliminación. En la Universidad Industrial de Santander -UIS-, entomólogos y diseñadores Industriales, usan metodologías para el diseño científico de nuevas trampas para el control de los insectos. La metodología involucra análisis del estado del arte, etología de los mosquitos, consulta a expertos, determinación de especificaciones y propuestas de diseño de la trampa. Luego se evalúan con matrices para determinar los probables para su desarrollo y construcción de modelos funcionales. Posteriormente se procede al diseño experimental donde se plantean las hipótesis para validar experimentalmente factores de comportamiento de los insectos a estímulos de color, contraste, características superficiales de materiales, forma y tamaño, para definir qué aspectos son más favorables para captura, retención o eliminación de mosquitos. Es nuestro interés dar a conocer a la comunidad científica la metodología de diseño, mostrando la eficacia obtenida por las trampas Gravi trap, HomeTrap y TorreVigia. Una premisa importante es desarrollar productos sin fuentes de energía, mecanismos o dispositivos electrónicos. Gravi trap demostró mayor preferencia del insecto por el color rojo para el cuerpo de la trampa y negro para la entrada, HomeTrap presentó un 80% de captura/eliminación, con una estructura de sección cuadrada y fabricada con materiales biodegradables. Los resultados de las trampas anteriores se obtuvieron en laboratorio. Para TorreVigia que utiliza conceptos de las trampas anteriores, mostró en campo un porcentaje de captura del 60%, con una entrada abierta de mayor área, también capturó otras especies de insectos.

Keywords: TrapS, design, methodology, vector, control

PO407

DOCUMENTATION OF BITING MIDGES FROM SOME SELECTED AREAS OF PAKISTAN

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Midges (Diptera: Ceratopogonidae) are the smallest two-winged blood sucking flies of cosmopolitan distribution. Biting midges cause painful bites and irritation and also act as vectors of different pathogens in humans and animals. This study reports distribution of biting midges and its associated risk factors in selected areas (Faisalabad, Peshawar, Rawalpindi and Islamabad) of Pakistan for one calendar year. To this, end collection was done fortnightly during morning and evening times using light traps, transferred in the eppendorf tubes containing 70% ethanol, labeled and shipped to Molecular Parasitology Laboratory, University of Agriculture, Faisalabad, Pakistan for stereomicroscopic taxonomy. Meteorological data like humidity, temperature, rain fall and wind speed were also recorded from Pakistan Meterological Department for the study duration. Of 266 samples, 40 (15%) were *Culicoides pulicaris*, 80 (30%) were *Culicoides obsoletus* and 146 (54.8%) were *Culicoides imicola*. Highest prevalence (51.5%) of biting midges was found in Islamabad and lowest (0%) in Faisalabad. Spring and Winter seasons got highest (40.98%) and lowest (5.1%) prevalence. Highest prevalence is observed during high temperature (35-40°C); while, lowest prevalence is observed in Winter season (10-12°C). In heavy rain fall and high wind speed, their number decreases upto 35%. The study is just a maiden attempt to report probable vectors which might be carriers of emerging and re-emerging arthropod infections in Pakistan. Further investigation on the vectorial capacity of the arthropods is required.

Keywords: Cosmopolitan, Light trap, Vectorial capacity

PO408

A LONGITUDINAL STUDY OF ADULT CULICOIDES ACTIVITY INSIDE AND OUTSIDE LAMBING SHEDS OVER THE WINTER

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Within a five-year period two major Culicoides borne diseases of ruminants have swept through Europe: bluetongue virus serotype 8 (BTV-8) and Schmallenberg virus (SBV). The putative vectors of both viruses are Culicoides biting midge species within the Obsoletus group and Pulicaris group. Both diseases have caused high economic and livestock losses throughout Europe, and both managed to overwinter, reappearing the following season. Currently the exact mechanisms for overwintering are unknown. Little evidence for transovarial transmission from adult midges to overwintering larvae exists and the activity of adult Culicoides is considered limited in season length (from April to November in the UK). This study aimed to investigate the winter activity of Culicoides biting midges inside lambing sheds in the south of England. A pilot study of 21 farms determined midges were active in lambing sheds during the 2015-2016 lambing period. The following winter, from November 2016 to April 2017, a longitudinal study was undertaken on 4 farms both inside and outside the lambing sheds. Culicoides were found to be active throughout both winters inside lambing sheds. The most abundant species were all putative vector species, with female Obsoletus group Culicoides comprising 88.3% of total Culicoides caught. Parous Culicoides were caught every month except January and February. Gravid Culicoides were caught every month, with the exception of February. This provides strong evidence for ongoing adult Culicoides activity throughout the winter, and therefore demonstrated the potential for ongoing virus transmission throughout the winter.

Keywords: Culicoides, overwintering, Schmallenberg virus, bluetongue virus

PO409

PERFORMANCE OF TWO STICKY TRAPS TO MONITOR RESTING AEDES INVASIVE SPECIES (DIPTERA; CULICIDAE) IN ITALY

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Among invasive Aedes species spreading in Europe, *Aedes albopictus*, *Ae. japonicus* and *Ae. koreicus* are currently present in Italy. Collection methods used for their sampling are mainly based on ovitraps to collect eggs and BG-Sentinel to catch host-seeking mosquitoes, while effective tools to collect blood-fed resting females are currently unavailable, despite the interest in studying the species feeding behaviour. We here present a comparative evaluation of the effectiveness of two sticky devices developed for Anopheline mosquitoes, Sticky Trap (ST) and Sticky Resting Box (SRB), to collect resting and blood-fed females of the two Aedes species. The study was performed both in the field, in an area where the two species are sympatric (Belluno province), and in the semi-field, *i.e.* in a green-house where 30 fed females of each species were contemporarily exposed to the two traps. In

the green-house, the two traps showed non-significant differences for both species (three replicates; median rates: SRB-*Ae. albopictus* 5%; SRB-*Ae. koreicus* 6%; ST-*Ae. albopictus* 17%; ST-*Ae. koreicus* 3.5%; Kruskal-Wallis P=0.44). In the field, however, ST collected higher number of females than SRB (mosquito/trap/day: SRB-*Ae. albopictus* 0.52; SRB-*Ae. koreicus* 0.04; ST-*Ae. albopictus* 5.74; ST-*Ae. koreicus* 0.30; Kruskal-Wallis P <0.0001) and no blood-fed *Ae. koreicus* females were sampled with SRB (*Ae. albopictus* in ST=39; in SRB=1). Results showed that both devices may effectively collect the two target species, although the very low *Ae. koreicus* densities outdoors did not allow to definitively assess the effectiveness of SRB in the field. Due to the rapid expansion of *Ae. koreicus* and its establishment in new territories, this data could be used to obtain new information about its ecology and the traps should be employed as additional tools to improve the surveillance system of invasive mosquito species.

Keywords: *Aedes albopictus*, Aedes koreicus, traps comparison

Vector and Pathogen Biology

PO410

CO-CIRCULATION OF *LEISHMANIA INFANTUM* AND PHLEBOVIRUSES FROM SANDFLIES IN THE BOLOGNA PROVINCE, ITALY

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Sandflies are tiny insects, often living close to domestic animals and humans. Females of these insects are hematophagous and their bites occur especially at night. Sandflies are poor fliers, flying silently for short distances, and with activity peaks during summertime. Sandflies are widely distributed in peri-Mediterranean countries and are vectors of many pathogens, particularly, protozoa of the genus *Leishmania* and some arthropod-borne viruses of the genus *Phlebovirus*. These pathogens often show an epidemiological link, sharing the same sandfly vectors: *Phlebotomus perniciosus* and *Phlebotomus perfiliewi* are able to transmit *Leishmania infantum* and Toscana virus (TOSV) in Italy. The co-circulation of phleboviruses and *L. infantum* was detected by specific Real-Time PCRs in sandflies sampled in two sites localized in Valsamoggia municipality (BO), Emilia-Romagna region, Italy. Sandflies were sampled with attractive carbon dioxide traps from June 2017 to September 2017. A total of 28,283 sandflies were collected (site-1: 19,441, site-2: 8,842), about 5% of these were identified at species level of the genus *Phlebotomus* achieved by morphological identification of males: *P. perfiliewi* (1,358, 98%) and *P. perniciosus* (30, 2%). One hundred ninety-four pools of 50 females were tested; *Leishmania* was detected in 60 pools (site-1: 38, site-2: 22), while, 63 pools tested positive for phleboviruses (site-1: 40, site-2: 23). Interestingly, 16 of these pools were positive for both protozoa and viruses. Amplicons obtained by the pan-phlebovirus PCR were sequenced and were ascribable to: Corfou virus (2), Fermo-like virus (40), Ponticelli virus (7) and TOSV (4). In conclusion, co-circulation of *Leishmania infantum* and phleboviruses was confirmed by their contemporary presence in the surveyed sites. Their coexistence was likely due to a common vector, *Phlebotomus perfiliewi*, strongly incriminated as principal vector for its abundance in that area. Among phleboviruses only TOSV is known as human infectious agent, while the potential pathogenicity of other phleboviruses must be investigated.

Keywords: Sandfly, *Phlebotomus perfiliewi*, *Phlebotomus perniciosus*, *Leishmania infantum*, *Phlebovirus*, Toscana virus, Italy

PO411

ENTOMOLOGICAL SURVEY OF POTENTIAL MALARIA VECTORS IN NORTH-EAST ITALY

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Malaria is one of the most important infectious disease in the world. In Italy it was eradicated more than 60 years ago. However, in 2017 unexpected cases in people with no travel history in endemic areas were reported in Italy, one of them fatal. Following this last case, an entomological survey was carried out in the areas frequented by the patient. In addition, Anopheles species collected in the frame of the entomological surveillance for West Nile virus were identified by morphological and molecular analyses. The aim of our study was to identify the population of *Anopheles maculipennis* complex present in north-east Italy in order to evaluate the presence of malaria vectors. Mosquitoes were collected from May to October 2017 using CDC-CO_2 trap and manual aspiration. In order to identify different subgroup species of *Anopheles maculipennis* complex a Real-Time PCR (max five mosquitoes per capture) and sequencing were performed. Only *Anopheles maculipennis* s.l. were caught by aspiration in the place of the human case (five *An. maculipennis* s.s. and two *An. messeae*). In total, 1349 *Anopheles maculipennis* s.l. were collected and 252 of them were identified; among them, 196 were *An. messeae* (n= 77.7%) and 56 *An. maculipennis* s.s. (n= 22.2%). Most of the mosquitoes were captured in Verona and Rovigo Provinces where farms and paddy fields are present that could explain the presence of zoophilic species (*An. messeae* and *An. maculipennis* s.s.). According to our data, the main vectors of malaria (*An. labranchiae* and *An. sacharovi*) are absent or present in very low density and autochthonous transmission is likely negligible. However, an entomological surveillance specific for Anopheles with collection of larvae and adults by aspiration in suitable places is needed.

Keywords: Anopheles, malaria, mosquitoes

PO412

INTRAESPECIFIC COMPETITION IN *AE. AEGYPTI* AND *AE. ALBOPICTUS*: EFFECTS ON THE PROPORTION OF MALES AND FEMALES, SIZE AND LOCOMOTOR ACTIVITY

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Aedes aegypti and *Aedes albopictus* are species of mosquitoes known for transmitting arboviruses to humans worldwide, such as dengue, chikungunya and Zika. These species can occur in sympathy in several areas of the world, colonizing the same types of breeding sites. The knowledge of the biology of *Ae. aegypti* and *Ae. albopictus* is essential for the understanding of the transmission dynamics of the pathogens they can harbor and for the targeting of control strategies. The objective of this study was to determine the effect of intraspecific competition on the proportion of emergence of males and

females of *Ae. aegypti* and *Ae. albopictus*, as well as in the size and locomotor activity of virgin females of both species. Both *Ae. albopictus* and *Ae. aegypti* eggs were obtained from established laboratory colonies. Intraspecific competition was evaluated under low (20 larvae - 20L) and high density (100 larvae - 100L), in basins containing 500 ml of water and 0.20 g of fish food. The larvae were monitored daily until the pupae stage. All pupae were individualized and mosquito sex was determined after adult emergence. Experiments of locomotor activity were conducted with virgin females of both species under laboratory conditions, using an activity monitor that registers individual activity every thirty minutes. After the activity, the wing of the females was measured to determine the size of the specimens. For the density of 20L, 2 of the 4 basins of *Ae. aegypti* showed higher numbers of emerged males, while the majority of *Ae. albopictus* presented an equilibrium in the proportion of both sexes. For 100L, both *Ae. aegypti* and *Ae. albopictus* presented a higher proportion of emerged males. In addition, for both species, the size of the wings was significantly different between the two densities, but no differences in locomotor activity were observed. Intraspecific competition in the densities tested seems to affect the proportion of males and females emerged and the size of these adults, but not the locomotor activity of females of both species.

Keywords: *Aedes aegypti*, *Aedes albopictus*, competition, emergence, size, locomotor activity

PO413

PARITY AND GONOTHROPHIC DISCORDANCE IN *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE) IN THE CITY OS SÃO PAULO, SP, BRAZIL

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Aedes aegypti and *Aedes albopictus* are two mosquitoes of major concern in Public Health since both species transmit arboviruses such as dengue, Zika and chikungunya to human during blood-feeding. In addition to the contact with the vertebrate host, longevity and gonotrophic discordance are important aspects in the determination of a successful vector. The objective of this study was to evaluate parity, presence of blood in the stomach and gonotrophic discordance in *Ae. aegypti* and *Ae. albopictus* captured in two areas in the city of São Paulo, SP, Brazil. Captures were carried out monthly, by aspiration, from January 2015 to August 2017. All females of *Ae. aegypti* and *Ae. albopictus* had their stomachs and ovaries dissected in the laboratory to determine the presence of blood and parity/maturation stage. Regarding to parity, most females of *Ae. aegypti* and *Ae. albopictus* were parous or had ovaries in late stages of development. Additionally, 36% and 27% of *Ae. aegypti* and *Ae. albopictus* females respectively were in gonotrophic discordance, as well as most of these females contained blood in the stomach. These data suggest a frequent contact of these species with the vertebrate host, as well as indicate the conditions of the study areas, which favors the survival of these mosquitoes, increasing the possibility of becoming infected and transmitting the viruses they can harbor.

Keywords: *Aedes aegypti*, *Aedes albopictus*, parity, gonotrophic discordance

PO414

CHARACTERIZATION OF A CISTEINE PEPTIDASE (DCCATL1) FROM *DIAPHORINA CITRI*, A VECTOR OF THE HUANGLONGBING DISEASE

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Huanglongbing (HLB), is considered the most devastating disease of citrus plants and currently, there is no cure available. The HLB infection is associated with the gram-negative bacterium *Candidatus Liberibacter asiaticus* (CLas). CLas is transmitted by the Asian citrus psyllid (ACP) *Diaphorina citri*, in a circulative manner, when the insect feeds on plant floem sap. *Diaphorina citri* is member of the insect order Hemiptera, which has the cysteine peptidases as the most abundant proteolytic enzymes being involved in important roles. In this context, the aim of our study was the recombinant expression, enzymatic characterization, inhibition studies and gene expression analysis of a cathepsin L-like from *D. citri*. The cloning was performed in pET SUMO vector and recombinant expression in Rosetta (DE3) cells for 4 hours at 20°C. The protein was solubilized with urea 8M and the refolding process was done by dialysis at 4°C. Recombinant DCcatL1 was expressed with a molecular mass of 37 kDa. DCcathL1 catalytic activity was determined using the fluorogenic substrates Z-FR-AMC and Z-LR-AMC and the inhibition assays were performed using recombinant citrus cystatins CclemCPI-1, CclemCPI-2, CclemCPI-4, CsinCPI-2. To access DCcatL1 expression, *D. citri* body parts such as head, gut and carcass were analyzed by RT-qPCR. The purified enzyme was activated at pH 4,5 and the catalytic efficiency for Z-FR-AMC and Z-LR-AMC was determined. DCcatL1 showed a preference for Z-FR-AMC with a kcat/Km=63 M-1S-1. The enzyme was strongly inhibited by the cystatins tested, but the strongest inhibitor was Csin-CPI2 (Ki=4, 46 pM). The gene expression profile of DCcatL1 in different body parts showed a higher expression in gut, suggesting a digestive function for the enzyme. Our results pointed DCcatL1 as a promising target for the development of new control strategies against HLB, possibly using RNA interference or overexpression of citrus cystatins in citrus plants.

Keywords: *Diaphorina citri*, HLB (Huanglongbing), cysteine peptidase, cathepsin L

PO415

INTERACTION NETWORKS AND ARBOVIRUSES: BLOOD-MEAL SOURCES OF MOSQUITOES COLLECTED IN COASTAL ECOSYSTEMS FROM SAN BERNARDO DEL VIENTO (CÓRDOBA, COLOMBIA).

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Mosquito blood meals provide information about the feeding habits and host preference of potential arthropod-borne disease vectors. Although mosquito-borne diseases are ubiquitous in the neotropical ecosystems, few studies in this region have assessed patterns of mosquito-host interactions, especially in modelling dispersion of emerging infectious diseases or during disease outbreaks. Based on collections made during 2011-2014 in a coastal Caribbean zone, we identified the source of 437 blood-meals from 9 species of mosquitoes from La Balsa, a rural location in San Bernardo del Viento – Colombian Caribbean. A PCR based method targeting two distinct mitochondrial targets and subsequent DNA sequencing was used in an effort to delineate vector-host relationships. A high richness in avian fauna dominated the assemblage of mosquito blood meals while wild mammals only represented a small portion of the blood meal pool. Most mosquito species fed on a wide variety of hosts, but anthropophily was extremely low with exception of *Ae. aegypti*. Bray-Curtis index and dendrogram analysis indicates that eight of nine mosquito species utilize hosts at different proportions and significative overlap among host species. The records of somes blood meal sources indicate possible reservoirs for WNV, SLEV, VEEV and DENV; additionally interactive networks shows evidence about the complexity in viral traffic in sylvatic cycles, which may have implications for the role of vertebrate community species in maintain patterns of enzootic circulation and determined in the future possibles emergence scenarios.

Keywords: Blood meal, Cytochrome B, DNA barcode, Mosquitoes, Host, interaction network.

PO416

BLOOD MEAL SOURCES OF *MANSONIA TITILLANS* AND *PSOROPHORA FEROX* COLLECTED FROM LA PINTADA (ANTIOQUIA, COLOMBIA)

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The municipality of La Pintada (Antioquia, Colombia) shows circulating arboviruses as Eastern Equine Encephalitis virus (EEEV), previously reported for this locality. Entomological research about mosquitoes in rural area evidenced a considerable abundance of *Mansonia titillans* and *Psorophora ferox*, probable vectors to EEEV. The aim of this study was to investigate blood meal sources of mosquitoes belonging to this area identify possible associations with host/reservoirs to arboviruses. Six species of blood hosts of 42 engorged adult female mosquitoes were identified using PCR assays with universal primers set based on cytochrome b mitochondrial DNA, sequencing and BLASTN tool allowing the molecular identification of birds, rodents, domestic animals, and humans. Mosquitoes were captured using a manual aspirator and CDC traps. Any feeding preferences were observed in two species studied and our results highlight the opportunistic feeding habits of the female mosquitoes in this study.

Keywords: Blood meals, Mosquitoes, Cytochrome B, Host vertebrate, arboviruses.

PO417

EFFECTS OF TEMPERATURE CHANGES ON THE LIFE CYCLE OF THREE MOSQUITO SPECIES

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Mosquito-borne diseases are an important public health problem, causes morbidity and mortality worldwide. Their biology and disease ecology are strongly related to environmental conditions such as temperature. This study investigated the differential effects of temperature on the duration of each life stage of *Aedes aegypti* (*Ae. aegypti*), *Anopheles stephensi* (*An. stephensi*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*). Adult mosquitoes were reared at a temperature 28±2ºC, relative humidity 70% ±10, in entomology laboratory. Egg laying and hatching time for *Ae. aegypti* was 180 to 220 hours at room temperature 25ºC and 160 to 170 hours inside the incubator at 28ºC. However, for *An. stephensi* ranged from 82 to 90 hours and 72 to 75.5 hours and for *C. quinquefasciatus* 80 hours and 70 hours at room temperature and incubator respectively. Temperature variation effect directly on developmental stages and high rate of development was observed in incubator as compared to room temperature. From the present study, it can be concluded, that global temperature variation results in greater deviations of average time span of mosquito life cycle. In next decades, the life cycle time span will decrease alongwith shorter development period and different morphological adaptations.

Keywords: *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*, Temperature, life stages, dengue, climate change

PO418

VARIATIONS IN THE ABUNDANCE AND ACRODENDROPHILIC BEHAVIOR OF *ANOPHELES (KERTESZIA) CRUZII* DYAR & KNAB, 1908 (DIPTERA: CULICIDAE) IN SITES WITH DIFFERENT PROPORTIONS OF FOREST COVER IN THE ATLANTIC FOREST, BRAZIL

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Introduction: The species *Anopheles cruzii* is an acrodendrophic mosquito that usually lives and feeds on hosts in the treetops, but eventually feeds on human blood at the ground level. This species is highly abundant in the Atlantic Forest developing exclusively in bromeliads and is considered the main vector of simian and human malaria in this biome. We investigated whether there are variations in the abundance and acrodendrophilic behavior of *A. cruzii* in sites with different proportions of native forest cover in an area with autochthonous cases of malaria located in the city of São Paulo, Brazil. *Methods:* Collections of adult mosquitoes were monthly carried out from March 2015 to March 2017 in five sites with different proportions of forest coverage (considering a buffer of 1km around the collection point). The collected specimens were morphologically identified. Variations in the abundance and acrodendrophilic behaviour of *A. cruzii* were analyzed using Generalized Linear Models and chi-square test for equality of proportions. *Results:* During the study period a total of 5,412 specimens were collected. A positive predictive relationship (p=0.03) between *A. cruzii* abundance and forest cover proportion was observed. The acrodendrofilic behavior showed a statistically significant variation between environments with lower and higher forest cover (p<0.001). In sites with more than 90% of forest cover, were collected 3.7 times more mosquitoes at the treetops than in the ground level. In sites with less than 75% forest cover were collected only 1.6 times more mosquitoes in treetops than in the ground level. *Conclusions:* Our results suggest that the loss of native forest cover and the consequent increase of anthropic influence lead to a reduction in the abundance of *A. cruzii*, but on the other hand an increase in the average frequency of this species in the ground level was observed.

Keywords: Abundance, acrodendrofilic, *Anopheles cruzii*, Atlantic Forest.

PO419

CORPORA ALLATA-SPECIFIC GENE EDITING IN MOSQUITOES: A GREEN CA REVEALS INTERESTING SECRETS

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Juvenile hormone (JH) is synthesized by the corpora allata (CA). *In vitro* approaches were used to identify factors responsible for the activation and suppression of JH synthesis in mosquitoes. We are interested in developing tools to study the role of regulatory factors *in vivo*. A system to drive CA specific expression will facilitate this type of experiments. Juvenile hormone acid methyl transferase (JHAMT) is a JH biosynthetic enzyme specifically and highly expressed in the CA. Using the CRISPR/Cas9 system, we have integrated a promoter-less gene encoding the green fluorescent protein (GFP) into the JHAMT locus. Transgenic mosquitoes show specific GFP expression in the CA. Analysis of transgene expression and survival of homozigous mosquitoes are revealing exciting data that uncover surprising aspects of CA functions. In this poster we will address two intersting questions: Is mosquito postembryonic development initially independent of JH? Do the two CA glands work always in synchrony? Recently we have integrated the gene SWITCH (inactive form of GAL4) into the JHAMT locus. Inducible UAS/SWITCH system will allow conditional expression in CA using RU486. This system will allow the CA-specific and inducible expression of selected genes or dsRNAs.

Keywords: Mosquito, corpora allata, gene editing, CRISPR, Juvenile hormone

PO420

SPATIAL AND TEMPORAL VARIATIONS IN THE ABUNDANCE OF *CULEX (MICROCULEX) PLEURISTRIATUS* LUTZ, 1903 AND *CULEX (MICROCULEX) IMITATOR* THEOBALD, 1903 IN AREAS WITH VARIATION IN PROPORTION OF VEGETATION COVER IN THE CITY OF SÃO PAULO, BRAZIL

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Introduction: Microculex is a subgenus of mosquitoes (Diptera: Culicidae) belong to *Culex* genus, restricted to the Americas and little studied, their role as pathogen vector is unknown. Their species are typically found in wild environments developing mainly in bromeliads, but some species of this group, such as *Cx. pleuristriatus* and *Cx. imitator*, can be found developing in urban green areas. Objectives: We investigated the spatial and temporal variations in the abundance of *Culex pleuristriatus* and *Cx. imitator* species in three areas located in the city of São Paulo, Brazil. Methodology: The sites selected for the study have different proportions of native vegetation covering a buffer of 1 km around the collection point (60%, 70%, 90%). The collections were carried out monthly, from March 2015 to April 2017. Immature forms were collected in bromeliads, bamboos and artificial containers. The specimens collected were morphologically identified in the laboratory. To evaluate the temporal variation, rainfall and monthly temperature data were obtained. Data analysis was performed using non-parametric statiscal tests of Kruskal-Wallis and Spearman's correlation. Results: 418 specimens of *Culex pleuristriatus* and 210 *Cx. imitator* were collected throughout the study. *Cx. pleuristriatus* was more abundant at the site with lower vegetation coverage ($p=0.04$), but did not show temporal relation with temperature and precipitation ($p>0.05$). For *Cx. imitator* we did not observed differences in abundance between environments and no temporal correlation with climatic variables. Conclusion: Our data showed that *Cx. pleuristriatus* abundance was influenced by variations in vegetation coverage, whereas *Cx. imitator* was not affected by such variations. We have not observed a direct relationship between abundance with temperature and precipitation for these species. Our future investigation will try elucidating other aspects of ecology, biology, and taxonomic relations of this subgenus.

Keywords: Microculex, ecology, mosquitoes, vegetation.

PO421

WEST NILE VIRUS IN ROMANIA

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The circulation of West Nile virus (WNV) takes place in cycles between mosquitoes and birds as main hosts, and the mammals including humans as tangential hosts. The WNV circulation was documented in Romania beginning with the '50s by serological investigations on healthy humans and domestic animals and the confirmation of this virus as etiological agent of sporadic and epidemic human neurological infections. An outbreak of more than 800 human cases of WNV neurological infections appeared in South Romania in 1996. This was the European signal of the increase of WNV circulation especially because of the global environmental changes including climatic ones. The WNV neurological infections continued to appear yearly after the outbreak in Romania on more extended areas. The multidisciplinary investigation using entomological, immunological, virology and molecular biology techniques have been performed in 2001 – 2017 on large territories in Romania on the main elements of the transmission cycles of WNV involving mosquito vectors, domestic and wild birds and horses in natural and anthropic ecosystems and their variations in correlation with changing environmental factors. The virus was detected in females of *Culex pipiens*, *Coquillettidia richiardii*, *Ochlerotatus caspius* and *Anopheles maculipennis* s.l. species. Males of *Culex pipiens* and also over wintering females of this species have been positive for the virus. The isolated viral strains were molecularly characterized. It has been demonstrated the intensive and permanent circulation of WNV on extended territories in the country and the permanent risk of its transmission to humans. The risk areas have been mapped. The permanent surveillance of West Nile virus endemic circulation and the implementation of integrated mosquito control programmes in the key areas at risk in Romania are adequate decisions for public health.

Keywords: West Nile virus, mosquito vectors, vertebrate hosts, Romania

PO422

USING CODON USAGE BIAS TO INVESTIGATE THE ROLE OF ALTERNATIVE HOSTS IN THE SPREAD OF ZIKA

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In the last years, outbreaks of Zika virus (ZIKV) and Dengue have been reported from both endemic and newly invaded areas of the world. ZIKV is in general associated with, and is well adapted to, primates and mosquitoes vectors, on which it can complete its cycle. There is insufficient information regarding other animal reservoirs and amplification hosts, but there's a concrete possibility that ZIKV can infect other species, including domestic animals both from endemic and newly invaded temperate areas; such knowledge is fundamental to properly describe the epidemiology of ZIKV and plan its management. Unfortunately, large scale screenings of putative alternative vectors are extremely time and cost consuming. We suggest to employ Codon Usage Bias (CUB, the uneven use of synonymous codons) to predict if alternative hosts, in particular those associated with humans, can vehicle ZIKV. This will be performed by contrasting codon usage of ZIKV of hosts deduced from transcriptomes/proteomes, calculating parameters like RSCU (relative synonymous codon usage), CAI (codon adaptation index) and ENC (effective number of codons). Our first results indicate that Zika has putatively poor replication efficiency in *Culex pipiens*. Ultimately, we advocate the analyses of CUB to understand evolutionary dynamics of ZIKV and other flaviviruses and better forecast their future outbreaks.

Keywords: Culex, Zika, viruses, codon usage, vectors

PO423

CHANGES IN THE CEMENT AGGREGATION-CONE PROTEOME DURING THE FEEDING PROCESS OF THE ADULT TICK *HYALOMMA LUSITANICUM* KOCH, 1844

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Ticks pose as the most relevant ectoparasites of veterinary importance in Mediterranean and Tropical regions, for their direct blood spoliation on hosts and ability to act as pathogen vectors. Upregulation of salivary proteins expression, during feeding, expedites blood up-take and evades hosts' rejection. Tick salivary glands (SG) produce a broad range of bioactive molecules responsible for anti-haemostasis, inflammatory response suppression and for immune modulation. By excreting excess water and ions accumulated during feeding, SG secure osmoregulation and concentrate huge amounts of blood up in a single meal. Hard ticks fixation-mechanisms to hosts dwell on a cement-like plug, secreted by the tick salivary glands. Attachment comparisons between different tick species revealed differences in the position and shape of such a rapid hardening substance. Sheer quantities of proteins are difficult to assign to particular functions such as cement formation. Nevertheless, to address such predicament, sequential expression patterns of the cement plug matrix of the tick *Hyalomma lusitanicum* are herein addressed. At the present study, cement collections, obtained both *in vivo* and *in vitro* (artificial feeding by silicon membranes), at 5 sequential time points: 1) unfed ticks; 2) early-feeding; 3) medium engorged; 4) detachment period and 5) drop-off; enabled the identification of several proteins from the cement proteome by mass spectrometry. Proteins were assembled accordingly to their gene ontology, showing different salivary products being produced during the progressive feeding moments. Microscopic observation of bovine biopsies collected at different sampling times of infestation allowed skin lesions characterization. In the light of the associations found between protein subsets and feeding phases, insights into cement plug assembly and formation are here discussed. Understanding such molecular events may lead to development of new control strategies.
Funded by Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Project UID/CVT/276/2013 (CIISA)

Keywords: *Hyalomma lusitanicum*, cement-plug, proteome, histopathology

PO424

MORPHOLOGICAL VARIATION IN THE HIGHLAND BONT TICK, *AMBLYOMMA POMPOSUM* DÖNITZ, 1909

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Infesting most of the world cattle population, hard ticks are the ectoparasites of livestock with the highest economic impact. Tick infestations are a major problem for animal production in tropical areas where prevention and control remain deficient. In Angola, cattle breeding is an economically relevant activity at both family and national levels. The highland bont tick, *Amblyomma pomposum* Dönitz, 1909, is widely distributed within the country. In this species the ornamentation pattern may present variations. In the present study, ornamentation differences perceived among specimens collected at Huambo, Angola, allowed the definition of groups of similarity. Within these groups, the morphology was examined, particularly focusing on specific characters, namely: idiosoma conformation; scutum or conscutum (shape, enamel ornamentation, punctuation pattern, festoons and shape of posterior margin in females); Coxae I spurs conformation; pulvilli; palps; female porose areas; spiracular plates; cervical pits, fields and grooves. The female genital apertures and spiracles were later dissected and treated with lactophenol solution and mounted on microscope slides for comparison. In addition, the partial nucleotide sequences from the genes coding for the 12S, 16S and COI from specimens belonging to each of the defined groups were amplified and sequenced. Retrieved sequences were equal, supporting that the specimens studied belong to the same species, confirming a naturally wide morphological variation at an intra-species level.
Funded by Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Project UID/CVT/276/2013 (CIISA)

Keywords: *Amblyomma pomposum*, Huambo, morphology

Dermanyssus Gallinae: An Endless Threat to the Industry and Human Health

PO425

EFFECT OF A-BIOTIC FACTORS ON THE VIRULENCE OF INDIGENOUS *METARHIZIUM* SPP. STRAINS AND COMMERCIAL PRODUCT AGAINST THE POULTRY RED MITE AND NORTHERN FOWL MITE

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Recently, high level of pesticide residues were reported in eggs in several countries in Europe, which resulted in extermination of contaminated eggs. Nowadays, pest management in poultry houses relies solely on non-specific, high residual pesticides which ultimately leak to food products. These pesticides are becoming more and more ineffective due to development of resistant in the pest populations and the difficulty of delivering it effectively. Entomopathogenic fungi based products have the potential to be successfully integrated into pest management of pests including mites. Here we focused on two major pests: Red Poultry Mite, *Dermanyssus gallinae* and Northern Fowl Mite, *Ornithonyssus sylviarum*. The direct damage from these pests is reduction in yield and egg quality but also they harm the welfare of laying hens. In the current study we first isolated indigenous strains of entomopathogenic fungi from natural populations of Red Poultry Mites and later assess the efficacy of entomopathogenic fungi products in poultry house against both mites. The strains isolated from mites were identified and classified using multi gene phylogenetic analysis. Isolates belonging to the *Metarhizium complex* were examined in a controlled environment bioassay against the Poultry Red Mite under optimal conditions and under poultry house conditions. Results indicated a significant effect of environmental conditions on fungi efficacy. Additionally, application of commercial product based on entomopathogenic fungus was ineffective in reducing Red Poultry Mites population when applied directly on the cages, but was highly effective in reducing Northern Fowl Mites.

Keywords: Entomopathogenic fungi, Poultry red mite, Northern fowl mite, Microbial control

PO426

ACARICIDAL AND REPELLENT EFFECTS OF *CNIDIUM OFFICINALE*-DERIVED MATERIAL AGAINST *DERMANYSSUS GALLINAE* (ACARI: DERMANYSSIDAE)

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The acaricidal activity of material derived from the rhizome of *Cnidium officinale* against *Dermanyssus gallinae* adults was investigated. The methanol extract of *C. officinale* exhibited 100% acaricidal activity after 48 h of treatment at a dose of 4,000 ppm. The acaricidal constituents of the plant were sequentially partitioned with several solvents and then purified using silica gel column chromatography and HPLC. (Z)-ligustilide from *C. officinale* was identified by GC-MS and NMR spectroscopy. Acaricidal activity was examined in three experimental tests (spray, fumigation and contact), and the spraying method was the most effective. The methanol extract of *C. officinale* showed both contact and fumigant activity, although only fumigant activity was observed with (Z)-ligustilide. However, the fumigant effect of the methanol extract and (Z)-ligustilide caused 86.5% and 62.6% mortality against *D. gallinae* adults at 48 h, respectively. These results suggest that *C. officinale*-derived material can be used for the development of a *D. gallinae* control agent.

Keywords: *Dermanyssus gallinae*, *Cnidium officinale*, acaricidal activity, (Z)-ligustilide

PO427

EVALUATION OF SUSCEPTIBILITY OF RED POULTRY MITE, *DERMANYSSUS GALLINAE* (ACARI: DERMANYSSIDAE) IN FIVE REGIONS TO 11 ACARICIDES

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The susceptibility of *Dermanyssus gallinae* adults, collected from poultry farms in 5 regions, to 11 acaricides was investigated. When bifenthrin and formic acid with pyridaben were diluted 100 times, bifenthrin showed 100% acaricidal activity; however, formic acid with pyridaben showed less than 20% acaricidal activity for poultry farms in 3 regions (Gyeongju, Chilgok, and Geumsan) except Yeoncheon and Anseong. The carbamate compound, carbaryl showed 100% acaricidal activity in most of the regions, except Gyeongju. A phosphorus compound, dichlorvos showed 100% activity in most of the regions, yet 66.7% acaricidal activity in Yeoncheon. The susceptibility of *D. gallinae* from poultry farms in Yeoncheon and Anseong to most acaricides was high; however, in the other farms, the susceptibility of *D. gallinae* varied depending on the acaricide. Clothianidin, thiamethoxam, fenitrothion, and formic acid with pyridaben showed differences in acaricidal activity among regions. Therefore, farmers should concentrate during the selection of these acaricides. However, carbaryl, cartap hydrochloride, dichlorvos, and bifenthrin showed high activity against *D. gallinae* collected from poultry farms in five regions. Therefore, these acaricides could be used in most of these regions. To control *D. gallinae* effectively, the alternation of acaricides is necessary, and indiscriminate pesticide use should be avoided. Therefore, this study can serve as a basis for controlling *D. gallinae*.

Keywords: *Dermanyssus gallinae*, Acaricide, Acaricidal activity, Susceptibility

PO428

MONITORING ACARICIDE RESISTANCE IN VARROA AND POULTRY RED MITE POPULATIONS FROM GREECE

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Varroa destructor, a major bee parasite bee and the poultry red mite (*Dermanyssus gallinae*) are very important problem in bee and poultry production, respectively, in Greece, as well as many parts of the world. Their control typically relies on acaricides, such as pyrethroids. However, the intensive use of acaricides may select for acaricide resistance. By using bioassays as well as molecular assays, we monitored the presence and frequency of insecticide resistance phenotypes and alleles in a number of parasitic mite populations from Greece. High levels of acaricide resistance were identified in some occasions, which were associated with the history of spraying application. The presence of known pyrethroid resistance mutations in the voltage-gated sodium channel of pyrethroid-resistant *Varroa destructor* was also recorded, at various frequencies. The results will help beekeepers and poultry farmers with their decision making as to when to include or exclude certain acaricide control products and thereby facilitate the application of effective and sustainable management programmes.

Keywords: Varroa, beekeeping, red mite, poultry, acaricide resistance, pyrethroid resistance mutations.

Session 11.
Morphology, Systematics and Phylogeny

Imaging Methods for Scientific Progress in Entomology

PO429

WING PATTERN VARIATION IN THE ALCON BLUE BUTTERFLY IN SCANDINAVIA OVER THE LAST 50 YEARS

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In 1964, Svend Kaaber carried out a large-scale survey of the morphology of the Alcon blue butterfly in southern Scandinavia, where he found clines in wing patterns, which he interpreted as evidence of two species interbreeding in the area (Kaaber 1964). The specimens from this study are still available, and we can now carry out more extensive image-based morphological analysis, as well as extracting DNA from them to also examine historical patterns of genetic diversity. We will present the initial results of image-based morphological analysis of Kaaber's specimens, plus those from more recent museum collections. In 2018 and 2019 we will revisit his collecting sites, and take new genetic samples using non-lethal techniques, as well as documenting current wing patterns. This will allow us to reconstruct the genetic and phenotypic history of the current populations, and to both test predicted effects of fragmentation and identify populations that are most vulnerable to extinction.

Reference

Kaaber, S. (1964) Studies on *Maculinea alcon* (Schiff.) -rebeli (Hir.) (Lep. Lycaenidae) with reference to the taxonomy, distribution, and phylogeny of the group. Entomologiske Meddelelser 32, 277-319.

Keywords: *Maculinea alcon*, Image analysis, population genetics, microsatellites, conservation

PO430

HITHERTO UNDESCRIBED CUTICULAR POUCHES OF RPW FEMALE GENITALIA, RECENT FINDINGS AND INTERPRETATION ATTEMPTS

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Red palm weevil (RPW) infestations menace palms with historical, economic and landscape importance in many regions of the world. Despite human efforts, the pest is still undeterred by actual control actions. Seeking out symbiotic Bacteria repository associated with RPW female reproductive apparatus, we encountered two paired and possibly eversible cone-shaped pouches, which open on both sides of the membranes between the spiculum ventrale and vaginal base. Such structures, never observed previously, can be inflated and everted by injecting and pressuring the weevil with human physiological solution. Study by macrophotography, stereoscopy, bright field light microscopy, SEM and Cryo-SEM showed that the ventral cuticle of the cones is membranous and decorated with minute seta-like processes (microtrichia) plus scarce setae. The dorsal cuticle is somewhat sclerotized, with the exposed side pitted by single or grouped (2-20) apparent duct openings. Such openings should correspond to gland outlets, are served either by a pore-channelled sieve or by a long ducted end-apparatus on the body side of cuticle. Non-destructive investigations and high-resolution micro computed tomography 3D observations (HR-μ-CT,-400 nm resolution) of resting vaginal base and its content allows the study of the organ interrelationship within the reproductive system, from the common oviduct to ovipositor tip. μ -CT provided very detailed anatomical micrographs of retracted pouches, common oviduct, copulatory pouch, cuticle and membranes. Muscles are also recognized. These evidences show complex gross anatomy and morphology of the region, persuading to further clarify the possible function of the structure.

Keywords: *Rhynchophorus ferrugineus*, *Phoenix canariensis*, dactylifera, *Cocos nucifera*, *Elaeis guineensis*, urban ornamental

PO431

FLUID DYNAMICS AND *XYLELLA FASTIDIOSA* COLONIZATION OF INSECT VECTORS

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Xylella fastidiosa is a plant pathogenic bacterium that persistently colonizes the cuticular surface of the precibarium and cibarium of insect vectors. The degree of vector colonization by *X. fastidiosa* may vary, with populations in the spittlebug *Philaenus spumarius* (Aphrophoridae), for example, being 1-2 orders of magnitude smaller than those found in the sharpshooter vector *Graphocephala atropunctata* (Cicadellidae). We used micro-computed tomography (micro-CT) technology to explore the foregut and to build a 3D model to better understand the physical dynamics of fluid flow in the regions of the foregut of vectors that are colonized by *X. fastidiosa*. Different spatial distributions and geometrical arrangements of bacteria in the foregut of these two vectors were also modelled. The results show that *P. spumarius* could potentially host twice as many cells as *G. atropunctata*. Our conclusion is that fluid dynamics, and not cuticular surface available, is responsible in maintaining smaller bacterial population in the foregut of *P. spumarius*. Areas for bacterial colonization are also presented and forces that bacteria undergo were studied and compared. This study shows that micro-CT technology is an useful tool for assessing fluid dynamics and forces generated in the foregut of sap-sucking species.

Insect Morphology in the Age of Phylogenomics

PO432

A PILOSITY ANALYSIS OF WORKERS OF THE EUROPEAN RED WOOD ANT *FORMICA PRATENSIS* (HYMENOPTERA: FORMICIDAE) IN TURKEY

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The European Red Wood ant *Formica pratensis* (Hymenoptera: Formicidae) is one of the two wood ant species living in Turkey and is distributed only in the Thrace Region of the country. In the present study, we investigated four morphological characters on workers of 87 different *F. pratensis* colonies (N=870) sampled in the region in order to determine if the Turkish population of the species is represented with the hairy N morph or the less hairy P morph described earlier. The characters used were the maximum measurable head width (HW), the number of standing hairs on one half of occipital margin frontad to eye level and seen in dorsal view (NBH), the length of longest hair on occipital margin of head seen in straight dorsal view (LBH) and the number of standing hairs on extensor profile of hind tibia (NHTI). The comparison of the pilosity data with those reported for Europe populations of the species revealed no clear discrimination of N or P ecomorphs for Turkish population. However, Principal Component Analysis based on the pilosity data revealed two distinct groups, one represented with colonies sampled in the northern woodland parts and the other represented with colonies sampled in the central steppe region. Our data showed that the NBH values of workers in Turkish population were even lower than the less hairy P morph in European populations. The higher NBH and lower LBH values measured in the steppe region group compared to the woodland region group showed that the relatively lower hair number in Turkish population was compensated with the increase in hair lengths in response to more sun exposure in the steppic area.
This research was financially supported by the Scientific and Technological Research Council of Turkey (TUBİTAK, Project No, 212T118).

Keywords: *Formica pratensis*, pilosity, ecomorph, ant ecology

PO433

CONTRIBUTION TO THE STUDY OF THE FINE STRUCTURE OF THE EGG IN THE GENUS *PSEUDOMALLADA TSUKAGUCHI*, 1995 (NEUROPTERA CHRYSOPIDAE)

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Oviposition and egg morphology in Neuroptera have been the subject of studies which for the majority of the families have contributed significantly to the growth of knowledge on these aspects of their reproductive biology. Chrysopidae, green lacewings, is one of the richest Neuroptera families and also one of the most studied, due to the bio-ecological features of some species which have long aroused applied interest (biological control). Thus chrysopids have been the subject of much research also as regards the aspects mentioned above. The advent of the scanning electron microscope (SEM) has also allowed to considerably increase knowledge of the external morphology of the egg of green lacewings. Progressive improvements to the SEM, as well the perfecting of techniques of examining samples, now result in images of ever better quality. To date, observations are available for various genera and many dozens of green lacewing species; a relatively large number of them belong to the genus *Pseudomallada Tsukaguchi*, 1995 (Chrysopinae). In Europe this genus includes about two dozen taxa of specific or subspecific rank, some of which are difficult to discriminate and problematic as regards their systematic level: research into ootaxonomy by SEM could at least potentially help to resolve some uncertainties. The present preliminary work, based mainly on micrographs obtained using SEM, deals with the external morphology of the egg of some European species of *Pseudomallada*. The fine structure of the chorion surface, micropylar area, length of the stalk, and egg distribution during oviposition can be all useful elements for the above purpose. These observations are compared with the data already given in the literature and deserve to be extended, for this as well as other genera, to the largest possible number of taxa of species-group and also to more populations of each species from different geographic areas within the respective areal, to verify similarities or differences.

Keywords: Green lacewings, scanning electron microscope, egg structure, chorion surface, micropylar area, ootaxonomy

PO434

THE GALVAGNI COLLECTION (ORTHOPTERA, DEMAPTERA, BLATTODEA, MANTODEA, PHASMATODEA) AT THE MUSEO CIVICO DI ROVERETO

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The Museo Civico of Rovereto was founded in 1851 and hosts several art and science collections. Concerning Zoology, the entomology is noteworthy, consisting of the collection Halbherr (Coleoptera), the collection Tamanini (Hemiptera with several types) plus the recently acquired collection Galvagni (Orthoptera, Dermaptera, Blattodea, Mantodea, Phasmatodea). Antonio Galvagni (1924-2015) has been an eminent orthopterologist, whose work served as reference and stimulus for all the orthopterologists dealing with Mediterranean and European Fauna. He described 2 genera and more than 40 spp and ssp just among Orthoptera, plus several more taxa of Dermaptera, Blattodea and Mantodea. The collection consists of about 30.000 specimens mostly of identified, sorted and unsorted, plus unidentified material, all pinned in entomological boxes. The insects are accompanied by the library of Gavagni, consisting of about 1.000 volumes and a comprehensive collection of separates covering taxonomy and faunistics of Euro-Mediterranean Orthoptera, Dermaptera, Blattodea. The first step after acquisition was to freeze all the boxes to avoid infestations of insects pest. Then started the databasing of the identified specimens. The study of the collection will progress comparing study areas surveyed by Galvagni with current surveys in the same areas. The acquisition of this collection make possible new connections between the Museum and scholars, between the Museum and other institutions, vivifying the noteworthy collection built by Galvagni. The Museum becomes a reference point for Orthoptera studies, resulting attractive also for further donations such as the Fontana Orthopteroid collection to be acquired during the current year.

Keywords: Orthoptera, Dermaptera, Blattodea, Mantodea, Museo Civico di Rovereto, Galvagni entomology collection

PO435

Poster moved.

PO436

A MOLECULAR ANALYSIS OF WESTERN PALEARCTIC SPECIES OF *ANASTATUS* WITH A REVISION OF THE CATALONICUS SPECIES GROUP

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Anastatus Motschulsky (Hymenoptera, Chalcidoidea, Eupelmidae, Eupelminae) is an economically important genus of parasitoid wasps. Most members of *Anastatus* are egg parasitoids of various insect orders, but mainly Lepidoptera and Hemiptera, with a few species developing inside Diptera pupae including the tsetse flies. We are undertaking a species delimitation and discovery approach based on the analysis of a mitochondrial DNA sequence of the cytochrome c oxidase subunit I (COI), a nuclear gene and morphology. Our dataset includes mostly Western Palaearctic species and coalescent and phylogeny-based approaches were used to delimit the species. For a selected group of species, namely the catalonicus species group, we are providing a taxonomic revision that includes the description of several new species and one new synonymy. We are also clarifying the application of the name *Anastatus* catalonicus that was misinterpreted in the past. The revision is accompanied by numerous illustrations, descriptions and a detailed key to assist in the identification by the non-specialists. The correct identification of the species included in this species complex is highly relevant because some are potential biological control agents that have been misidentified in the past.

Keywords: Parasitoid wasp, taxonomy, DNA barcoding, species delimitation

PO437

FIRST MOLECULAR PHYLOGENY OF THE GENUS *PNIGALIO* (HYMENOPTERA: EULOPHIDAE) BASED ON MITOCHONDRIAL AND RIBOSOMAL GENES

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The genus *Pnigalio* Schrank (Hymenoptera: Eulophidae) includes over 60 species of ectoparasitoids of numerous insects, mostly leafminers and gall makers, belonging to the orders Coleoptera, Diptera, Hymenoptera and Lepidoptera. The taxonomy of this genus is challenging due to the high variability and phenotypic plasticity of putatively diagnostic characters, unavailability of many types and original descriptions based often on single specimens. As a consequence, many species are still poorly characterized and difficult to identify using the available dichotomous keys. The evolutionary relationships are also still unknown, as a comprehensive phylogeny is not available yet. We present the first molecular phylogeny of *Pnigalio*, based on three markers: a fragment of the mitochondrial gene COI (over 400 specimens sequenced), and the 28S-D2 (over 300 specimens sequenced) and ITS2 (over 200 specimens sequenced) portions of the ribosomal gene complex. Studied specimens were mostly collected from numerous host plant systems over a wide geographical range, mostly in the Palearctic region, but also include museum specimens, some of which belonging to type series. The concatenated phylogeny presented here resulted into: 1) a much higher diversity, often cryptic, than originally anticipated; 2) the identification of several species groups that can now be recognized based also on morphological traits; 3) ecological and evolutionary insights that will be discussed. This phylogeny represents the first step toward a comprehensive taxonomic revision of the genus *Pnigalio*.

Keywords: 28S, Chalcidoidea, COI, ITS2, systematics, taxonomy, parasitoids

PO437a

A TINY RELATIVE OF INSECTS: EFFECTS OF MINIATURISATION IN THE ANATOMY OF THE MINUTE SPRINGTAIL MESAPHORURA SYLVATICA (HEXAPODA; COLLEMBOLA: TULBERGIIDAE)

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Collembola, or springtails, are abundant soil microarthropods and part of a basal hexapod group. Many of them are less than one millimetre long, which makes them a good model for studying miniaturization effects in arthropods. The anatomy of insects has been analyzed in numerous studies, including recent research on microinsects, but data on the anatomy of collembolans remains scarce. Moreover, the anatomy of a collembolan species smaller than 1 mm long has never been described comprehensively. Therefore, in this study we analyze the anatomy of the minute springtail Mesaphorura sylvatica (Rusek, 1971) (body length 400 µm) for the first time. It is described using scanning and light microscopy and 3D computer reconstruction. Effects of miniaturization have been revealed after a comparative analysis of data from this study and from studies on the anatomy of larger collembolans, and are discussed in the comparison with such effects found in microinsects. Considerable changes in the nervous system have been observed, showing two pairs of apertures in the brain with three pairs of muscles running through them. The nomenclature for the musculature is analyzed and elaborated based on previous descriptions. A total of 22 pairs of muscles are found in the head, 53 pairs in the thorax, and 63 pairs and 1 unpaired transversal muscle in the abdomen. Asymmetry of reproductive organs, reduction of musculature (and absence of midgut musculature) and absence of any organs of the circulatory system are other indications of miniaturisation effects which were also found in microinsects. Unlike microinsects, M. sylvatica did not undergo the reduction of the salivary glands and endoskeletal structures of the head and thorax. Further research on minute springtails will improve our knowledge of miniaturization in living organisms and of the morphology of Collembola.

Keywords: Collembola, miniaturization, morphology

PO438

CRYPTIC SPECIES OR SPECIES POORLY (OR WRONGLY) STUDIED? THE CASE OF *GEOICA UTRICULARIA* (PASSERINI) (HEMIPTERA: APHIDIDAE: ERIOSOMATINAE: FORDINI)

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The concept of cryptic species is frequently applied to species that present morphological characters that make them indistinguishable from other related species. However, on many occasions, species that are poorly studied or from which few taxonomic or bionomic studies have been carried out are also wrongly qualified as cryptic. Aphids (Hemiptera: Aphididae) exhibit remarkably complex life cycles characterized by cyclic parthenogenesis. Within aphids, members of the tribe Fordini (Eriosomatinae), but also of Anoeciinae and Hormaphidinae, develop particularly complex life cycles with many different alternative morphs often including highly similar homologous morphs in different species. Thus, knowledge of their taxonomy, biology and phylogenetic relationships is scarce in many cases and sometimes equivocal due to the complexity, variability and plasticity of their life cycles but above all to the absence of serious taxonomic and bionomic studies. Among Fordini, the genus *Geoica* groups around 14 species, which in their zones of origin are holocyclic and heteroecious, being gall-dwelling in their primary hosts (*Pistacia* spp.) and root-dwelling in their secondary hosts (mainly Poaceae), but can develop anholocyclic populations in the latter. During years, species have been described either based on the gall- or on the root-dwelling morphs, but to date, in no species have all their morphs been correctly described, nor are their life cycles fully known. In Europe, to date, four species have been cited (*G. lucifaga* (Zehntner), *G. setulosa* (Passerini), *G. utricularia* (Passerini) and *G. wertheimae* (Brown & Blackman) that are considered to be perfectly defined. Applying an integrative taxonomy, morphological, bionomic and molecular studies of different species of Geoica have been carried out in the Iberian Peninsula, concluding that under the name of *G. utricularia* two different taxa have been considered and that the seasonal polymorphism that occurs in some species could be behind the different taxa assigned to the "utricularia" group.

Keywords: Galling aphids, phenotypic plasticity, Geoica, molecular markers

PO439

SYSTEMATICS OF TWO NEW GENERA OF *TINGIDAE* (HEMIPTERA: HETEROPTERA) FROM THE CRETACEOUS AMBER FORESTS OF MYANMAR

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Insects are one of the most abundant and diverse groups of animals today and this is thought to have been true throughout their evolutionary history. Tree resin fossilises as amber, trapping within it any unfortunate creature that happens to fall into it. The amber acts as a natural sticky trap, capturing insects and preserving a record of the past that we can use to reconstruct extinct ecosystems and phylogenetic relationships. Burmese amber is around 100 million years old and there are many thousands of insect inclusions that are currently being described and analysed. Here we present the results of one study in which two new genera of *Tingidae* are described based on a morphological character based phylogeny.

Keywords: Palaeoentomology, amber, Asia, morphology

PO440

PHYLOGENETIC ANALYSIS AND WING EVOLUTION OF MOSQUITOES (DIPTERA: CULICIDAE)

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Introduction: Mosquitoes (Culicidae: Diptera) comprise a monophyletic group, but some relationships within the family are not fully resolved. There are significant gaps in group knowledge, especially for Neotropical genera. *Objectives:* The aim of this study was to elaborate a phylogenetic hypothesis for the Culicidae family based on mitochondrial genomes. In addition, it was analyzed how wing shape evolved over time in these groups. *Methods:* We analyzed 1,429 wings of 76 different species covering 20 genera. For genetic analysis, sequences from mitochondrial genomes from 21 species were used. *Results:* The wing shape proved to be a good taxonomic marker, because the analyses in each tribe or genus were able to reveal natural clusters. The magnitude of the morphological divergence seems to follow a hierarchical pattern, where: subfamily >tribe >genus >subgenus >species >population. Regarding the mitochondrial genomes, the topology constructed with these data revealed well supported groups, and some were discordant to the current phylogeny of the family. Mitochondrial genomes generally followed the same genus order, except in the representatives of the Sabethini tribe, where there was a translocation of two transporter RNAs; this fact is unprecedented in Culicidae so far. The ratio between non-synonymous and synonymous mutations revealed that the mitochondrial genome of the Culicidae is under strong purifying selection. *Discussion and Conclusions:* The Sabethini tribe presented a unique grouping in both genetic and morphological analyzes. In general, morphology and genetics were inconsistent, and this may be occurring due to (a) distinct evolutionary rates between them, (b) any marker used to be under positive selection or (c) genetic canalization. Great support was found for some groups within Culicidae, such as Anophelinae and Sabethini tribe, and the mitochondrial genome proved to be a useful tool in the reconstruction of the evolutionary history of mosquitoes.

Keywords: Mitochondrial genome, wing geometric morphometry, translocation, evolution

PO441

PHYLOGENETIC STATUS OF *APANTELES GALLERIAE* WILKINSON (BRACONIDAE: MICROGASTRINAE) BASED ON CYTOCHROME OXIDASE-I

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Apanteles galleriae Wilkinson (Braconidae: Microgastrinae) is a larval endoparasitoid wasp species belonging to the Microgastrine subfamily used for the biological control of agricultural pest lepidopteran species. It is has been distributed in many different localities of the world. Phylogenetic status and haplotypes of *A. galleriae* rely on COI sequences determined. Local samples were collected from Rize-Turkey. Dneasy (Qiagen) kit for DNA extraction and Universal COI primer pair for PCR amplifications were used. PCR products were sequenced by ABI 3730XL Sanger Sequencer (Macrogen, Holland). For pyhlogenetic analysis, COI sequences of Sub-family Microgastriinae was downloaded from BOLD taxonomy browser. All COI sequences aligned by Clustal W. Evolutionary divergence between species were estimated by pairwise distance. Minimum Evolution (ME), Maximum Parsimony (MP), Maksimum Likelihood (ML), and Neigbor-Joining (NJ) trees were conducted at subfamily, genus, and species levels in MEGA7. Haplotype analysis of the COI sequences of *A. galleriae* was performed on the DnaSPv6.10.04. All genera of the Microgastrinae sub-family were distributed similarly at NJ, ML, and ME analysis. Genera *Apanteles*, *Paroptilis*, *Pholetesor*, *Hypomicrogaster*, *Snellenius*, *Larissimus*, *Rhygoplitis*, and *Kiwigaster* were clustered. As a result of NJ, ML and ME trees, *A. galleria* species has proven to be closest to *Apanteles pablovasquenzi*, *A. rolandovegai*, *A. brunnistigma*, *A. glenriverai*, *A. monicachavarriae*, *A. obscurus*, *A. glenriverai*, and *A. arielopezi* clustered in the same main branch with *A. galleria*. At haplotype analysis TR-ApGHap1-Rize was the closest haplotpye to NZ-ApGHap4-New Zelland, JQ853455.1. Our results would contribute to biodiversity and solution of controversies in phylogenetic relationships of *A. galleriae*.

Keywords: *Apanteles galleriae*, COI, Phylogeny, Rize-Turkey

PO442

INTEGRATIVE TAXONOMY OF POLISTINE PAPER WASPS REVEAL A RICH BIODIVERSITY IN NIGERIA

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Polistine paper wasps abound in Nigeria and observations suggest that the region is home to many undescribed species of social wasps. These social wasps perform ecological services such as pollination of agricultural crops, predators of pests of agricultural crops, bioindicators of environmental quality and biodiversity, and vector and natural reservoirs for brewers' yeast, *Saccharomyces cerevisiae*. In addition, social wasps are important models for the study and understanding of evolution of social behaviour in animals. The biodiversity of Polistine paper wasps in Nigeria is completely undescribed, despite their apparent high abundance and putative roles in the ecosystem. This knowledge gap constitutes an impediment for conservation, sustainable harnessing of the natural capital they offer and studies on any endemic West African species. To address this, Polistine paper wasps were collected from their nests across Nigeria and subjected to morphological and molecular identification. Molecular identification involving DNA barcoding was crucial in resolving species identity for morphologically cryptic species. Eleven species of social wasps were identified, from the three genera Belonogaster, Polistes, and Ropalidia, including four new undescribed species. The difficulties in amplifying and sequencing members of the three genera Belonogaster, Polistes, and Ropalidia with universal primers was overcome by designing a Polistine specific set of primers which amplified and sequenced approximately 620 bp COI gene in all the Polistines. Integrative taxonomy remains the best approach in species identification and is advocated for Polistine survey across all the biodiversity hotspots in Africa.

Keywords: Biodiversity, Nigeria, polistine, wasps

PO443

ANCESTRAL AND DERIVED HORN PATTERNS IN ONTHOPHAGINI DUNG BEETLES

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In Onthophagini dung beetles, sexual selection has generated a spectacular diversity in the head male weaponry. More in detail, in tunnelling species that display a guard behaviour of the female, the intensity of the male-male reproductive competition led to the development of weapons (secondary sexual traits) such as long horns or laminae, used to keep out the rival males from their territory by a face-to-face combat, but also in the male-female cooperation during the reproductive phase. In this framework, we studied the morphotypes of the extreme weaponry exhibited by males within a monophyletic clade of 15 Afrotropical Onthophagini species. In these taxa major males are characterized by a vertex carina (or horn) usually modified into a wide lamina. To identify potential evolutionary trends of the horn shape variation, we evaluated the phylogenetic relationships within the group obtained by using phenotypic external and internal characters. The dataset included the geometric morphometric partition with 12 different landmark configurations and the discrete partition comprehending 53 characters, combined into a single matrix processed by software TNT v.1.5. The unique fully resolved tree resulting from the combined phylogenetic analysis highlighted several clades, each characterized by a distinct horn pattern. The evolutionary trends of the horn shape thus allowed to identify ancestral and derived patterns in major males.

Keywords: Scarabaeinae, Phylogenetic relationships, Major males, Exaggerated Trait

PO444

THE EVOLUTION OF THE EPIPHARINX CHEWING SYSTEM IN CARRIER BEETLES

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Carrier beetles of the Eucraniini group, a small tribe with 4 genera endemic to Argentina, live in desert areas and are adapted to feed on dry, small mammal dung pellets. They grasp and lift pellets with the fore tibiae and move forward using only their middle and hind legs. This food-lifting relocation behaviour is considered a derived condition, probably evolved from a tunnelling behaviour. By using the SkyScan 1174 Micro CT, we studied the 3D morphology of the head of two closely related species, *Anomiopsoides cavifrons* (Burmeister, 1861) and *A. heteroclyta* (Blanchard, 1845). In both species, the clypeal armure consists of two central and two lateral (both symmetrical) processes, which are exaggerated to the point of modifying the entire structure of the head and the morphology of the mouthparts. Noteworthy is the case of the epipharynx (located on the lower surface of the head under the maxillae), with the epitorma producing a longitudinal enlargement on the fore edge, a sort of tooth that faces towards a small protrusion matching to a similar little tooth, present on the ventral surface of the clypeus near the anterior margin. The epipharynx plays a specific role in lifting and selecting food particles. These two coupled "teeth" can be functionally related to hash food before ingestion. The shape of the epipharynx chewing system seems therefore to be particularly adapted to manipulate small - and dried - dung pellets and/or constrained by the extreme development of the external symmetrical horns.

Keywords: Coprophagy, Mouthparts, 3D, Micro CT

PO445

DIRPHYS (HYMENOPTERA: APHELINIDAE) - GREGARIOUS ENDOPARASITOIDS OF WHITEFLIES (HEMIPTERA: ALEYRODIDAE) IN THE NEOTROPICS

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Dirphys Howard is a genus of aphelinid primary parasitoids of whiteflies (Aleyrodidae). It is the only known genus of obligate gregarious endoparasitoids of Aleyrodidae, and is known so far only from the Neotropical region. Until the current taxonomic revision, six described species were known. The current study describes an additional 16 species, bringing the total to 22. 10 of the newly described species are represented by holotype specimens that have had DNA extracted without damage to the sclerotized parts of the body, *i.e.* using a (so-called) "non-destructive" protocol. This extraction method, and the subsequent Sanger sequencing procedure, leave behind enough genomic DNA for future study, including

NGS, UCE, anchored enrichment, and other possible technologies that might be developed in the future. The workflow from field collection to open-access publication is described, which results in an online platform for the addition of new species in the future, maintaining an up-to-date, iterative, taxonomy. Matrix-based identification keys, which are comprehensively illustrated and can also be used to generate formal descriptions, provide the basis for the future of online taxonomic revisions. In this way taxonomy can be kept continually up to date, as well as universally accessible, and compliant with the International Code of Zoological Nomenclature.

Keywords: Cumulative taxonomy, iterative taxonomy, code-compliance, systematics online.

P0446

FEATURES ASSOCIATED WITH MINIATURIZATION IN THE WING APPARATUS OF THE FAMILY PTILIIDAE (COLEOPTERA)

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Miniaturization is one of the major directions in insect evolution. It is associated with morphological and physiological changes. It is known that some of these changes affect the wing apparatus. Most microinsects are characterized by ptiloptery: strongly narrowed wing blade fringed with long setae. The wing apparatus of Ptiliidae, the coleopteran family that contains the smallest free-living insects, has never been studied in detail, and the likely complete sequence of evolutionary transformations and adaptations associated with miniaturization in the wing apparatus of this family have never been comprehensively reconstructed. In this study, we describe the wings of several species of the family Ptiliidae, compare them with those of two species of Hydraenidae (the sister group to Ptiliidae), and analyze the likely evolutionary changes associated with miniaturization. In more primitive species of the family Ptiliidae, the wing contains at least five veins, a wide wing blade, and a marginal fringe of more than 300 setae, while the smallest species of the family have at most two veins, a narrow wing blade, and about 40 setae on the wing margin. The setae, which form most of the flapping surface, are brush-like, bearing peculiar outgrowths on their surface, which are denser towards the apex. These outgrowths increase the flapping surface of the wing and probably affect flight aerodynamics. The folded wing of the Ptiliidae is bent at four points. The wing-folding patches on the abdominal tergites play the main role in wing folding. The size and shape of the patches and the number of segments on which they are located directly depend on wing shape. The peculiar wing apparatus of ptiliids must be associated with special flight aerodynamics, the further study of which may help develop miniature flying devices.
This study was supported by the Russian Science Foundation (project no.№ 14-14-00208).

Keywords: Ptiliidae, wing, morphology

P0447

PHYLOGENY OF *ANTHOMYZIDAE* (DIPTERA) WITH EMPHASIS ON HOLARCTIC SPECIES IN LIGHT OF MOLECULAR DATING

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New phylogenetic hypothesis of the relationships of Holarctic species of the genera *Anthomyza* Fallén, 1823, *Epischnomyia* Roháček, 2006 and Arg*anthomyza* Roháček, 2009 (Diptera: *Anthomyzidae*) has been presented based on multigene analysis of the widened species dataset (against the previous paper Roháček & Tóthová 2014). The new molecular hypothesis is in good agreement with that of Roháček & Barber (2016) based on morphological data but it resolved relationships of some *E. Palaearctic* species affinities of which have hitherto been unclear. Timing of evolution of Holarctic *Anthomyzidae*, with special regards to most speciose genus *Anthomyza*, is discussed. The time of lineage divergences was calculated using Beast 1.8.3 and the results are compared with timing of existence of continental bridges between Palaearctic and Nearctic regions and their iterruptions during the Cenozoic Era (when the evolution of *Anthomyzidae* has been in progress) causing the spliting of taxa by allopatric speciacition (vicariance).

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Keywords: *Anthomyzidae*, *Anthomyza*, *Epischnomyia*, Argantomyza, DNA, molecular dating, Palaearctic, Nearctic

P0448

TO BE, OR NOT TO BE (A SENSILLUM): HAMMERING STRUCTURES IN PIMPLINAE UNVEILED

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Pimplinae are parasitoids belonging to the family Ichneumonidae that attack and develops inside hidden host, in most cases pupae of Lepidoptera. Female wasps evolved a peculiar host recognition strategy, termed as vibrational sounding, that involves the use of self-produced vibrations which are transmitted through the antennae on the substrate and perceived back as an echo using the leg subgenual organ. In this study we investigated, using both scanning (SEM) and transmission electron microscopy (TEM), the antennal tips of some species belonging to the Pimplinae, *i.e. Pimpla turionellae* L., *P. luctuosa* Smith, *P. nipponica* Uchida e *Xanthopimpla stemmator* (Thunberg). In all the investigated species, the antennal tips present peculiar apical pegs with different shape and number, often defining a flattened sole devoid of other antennal structures, such as sensilla. These pegs are present in both sexes with different number and development, are inserted on the antennal wall through an inflexible socket and present a cuticular shaft with cuticle of different thickness. We never found the presence of sensory neurons or glandular epithelium associated with these pegs, therefore we can exclude any sensory function. Because of their peculiar morphological features, we hypothesize for the antennal apical pegs a role in the context of host searching behavior (in the case of the female through the vibrational sounding strategy), as well as during mating behavior.

Keywords: Parasitoids, ultrastructure, antennae, sensilla, host recognition, vibrational sounding

PO449

WILL THE INTEGRATIVE APPROACH BRING LIGHT INTO THE PHYLOGENETIC RELATIONSHIPS OF SUBFAMILY LIMNOPHILINAE (LIMONIIDAE: DIPTERA)?

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Family Limoniidae includes over 10,000 species worldwide. Together with families Cylindrotomidae, Pediciidae, Tipulidae, and Trichoceridae belongs to infraorder Tipulomorpha. European authors divide the family into four subfamilies (Dactylolabinae, Limnophilinae, Chioneinae, and Limoniinae), while North American authors consider family Limoniidae as the subfamily of Tipulidae. Limoniid crane flies have slender body with long and narrow wings and long legs. Both their larvae and adults typically occur in moist environments, all around the world. The aim of this study is to reconstruct phylogenetic relations between selected genera of subfamily Limnophilinae, using multigene as well as classical (morphology) approach. The main focus is the question of the monophyly of this subfamily that has been challenged in several recent studies.

Keywords: Limnophilidae, phylogeny, DNA, morphology

PO450

THE EFFECT OF MINIATURIZATION ON THE ANATOMY OF *CORYNONEURA SCUTELLATA* (DIPTERA: CHIRONOMIDAE), ONE OF THE SMALLEST DIPTERANS

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Miniaturization, or decrease in the body size, is a widespread trend in the evolution of animals. It affects the structure of vertebrates and invertebrates, both aquatic and terrestrial, but the scope of changes related to the decrease in the body size is different in different taxa. In spite of the large biodiversity and wide distribution of the smallest dipterans, there are barely any detailed studies on the anatomy of these insects. The purpose of this study is to reveal the internal structure of *Corynoneura scutellata* Winnertz, 1846, one of the smallest dipteran species of the suborder Nematocera. Optical microscopy and 3D reconstruction were used to study the anatomy of this species. It is shown that, in spite of the considerable decrease in the body size, the majority of the organ systems retain their typical structures. Miniaturization has resulted in some simplification of the tracheal system: there are only a small number of subramose tracheae, with the air sacs undeveloped. The decreased the body size has also caused a simplification of the circulatory system, which consists only of a long thin aorta without any complementary vessels. We have revealed a considerable concentration ganglia of the central nervous system along with a reduction of some head muscles and the muscles associated with the gut. The reproductive system has not changed morphologically, but it is characterized by a very small relative volume in comparison with microinsects of other orders. This first-ever study of the anatomy of one the smallest dipterans considerably contributes to our understanding of the effects of that miniaturization on the internal structure of insects.
This study has been supported by Russian Science Foundation (project no. 14-14-00208).

Keywords: Miniaturization, Diptera, anatomy

Evo-Devo Perspectives on Insect Morphology

PO451

UNUSUAL “CEPHALIC PLACENTA” SUPPORTS EMBRYONIC DEVELOPMENT IN VIVIPAROUS DERMAPTERAN, *HEMIMERUS TALPOIDES*

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The Dermaptera order contains around 2200 insect species with diverse reproductive strategies. The vast majority of dermapterans are oviparous, *i.e.* the females lay eggs and the embryonic development takes place in an external environment. In contrast, species representing two epizoic groups (Arixeniidae and Hemimeridae) are viviparous, *i.e.* embryogenesis takes place inside the female, which gives birth to live offspring. Here, we demonstrate distinct adaptations for viviparity in embryonic and maternal tissues in *Hemimerus talpoides*. Our ultrastructural analysis revealed a novel mechanism of matrotrophy, which operates during oogenesis and involves characteristic modification of endoplasmic reticulum cisternae. Conspicuous and apparently inactive para-crystalline stacks of the endoplasmic reticulum, arising in the oocyte cytoplasm, are transferred into the embryos and become activated after commencement of embryonic development. This is the first report of the maternal investment in embryogenesis in the form of inactive but ready-to-be-used membranous organelles deposited in the oocyte to ensure unhindered development of the future embryo. Our morphological analysis also indicates that in Hemimerus, transformed follicular/ovarian cells, on the mother’s side, and an evagination of the dorsal vessel, on the embryo’s side, converge to form a cephalic vesicle, a structure analogous to a placenta. The cellular architecture of this unusual “cephalic placenta” points to its participation in an exchange of low molecular weight substances between the mother and the developing embryo.
This study was funded by a research grant OPUS 11 (UMO- 2016/21/B/NZ8/00560) from the National Science Centre, Poland.

Keywords: Viviparity, oogenesis, embryogenesis, matrotrophy, pseudoplacenta, Dermaptera, insects

PO452

ADAPTATIONS TO VIVIPARITY IN *ARIXENIA ESAU* (HEXAPODA, DERMAPTERA): AN EXCRETORY ASPECT

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Earwigs (Dermaptera) are one of a few insect groups in which more than one mode of reproduction occurs.The majority of dermapteran species are free-living and oviparous - females deposit eggs in the external environment and the entire embryonic development takes place under the protective coverings of the eggshell. However, in species representing two families of epizoic dermapterans: Arixeniidae and Hemimeridae, embryos develop inside a female, which gives birth to live larvae. In *Arixenia esau*, both embryos and the first instar larvae develop inside a mother's reproductive system. Such a reproductive strategy poses many physiological challenges for the mother, one of which is the removal of metabolic waste produced by the developing offspring. To elucidate how the Arixenia females cope with this challenge we have examined the larval excretory system. Our comparative analyses of the early and late first instar larvae revealed distinct modifications in the ultrastructure of the Malpighian tubules indicating that these organs are functional. This is also evidenced by an increasing number of vesicles containing spheroids in the epithelial cells of the Malpighian tubules. The results of the electron probe microanalyses suggest additionally that the larval Malpighian tubules are mainly involved in maintaining ion homeostasis. We also found that the lumen of the larval alimentary canal is occluded by a cellular diaphragm at the midgut-hindgut junction and that cells of the diaphragm and midgut accumulate metabolic compounds. Such an organization of the larval digestive system apparently prevents fouling of the mother's organism with the offspring metabolic waste and can be regarded as an adaptation for viviparity.

This study was funded by a research grant OPUS 11 (UMO- 2016/21/B/NZ8/00560) from the National Science Centre, Poland.

Keywords: Viviparity, development, Malpighian tubules, excretion, spheroids, Dermaptera, insects

Systematics, Biogeography and Ecology of Leaf Beetles - Third European Symposium on the Chrysomelidae

PO453

BARCODING EFFICIENCY ON CHRYSOMELIDAE OF EURO-MEDITERRANEAN AREA

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DNA barcoding is a useful approach widely adopted for insect identification through the use of the mitochondrial marker COI. Also for Leaf beetles (Coleoptera: Chrysomelidae), barcode libraries developed starting from accurate morphological identifications of individuals are efficient tools for recognizing species in absence of taxonomic expertise. In molecular species identification through barcode sequences, nucleotide distance-based approaches are usually adopted to define a limit between intra-interspecific level. A-priori fixed thresholds are commonly applied, but they are related to identification errors and thus a decrease of identification efficiency. In this study, an 889 sequences barcode library of Euro-Mediterranean Chrysomelidae was developed; the efficiency of DNA barcoding for Chrysomelidae identification was tested on it and on a further library composed of 7,237 sequences, including those already available in on-line repositories; moreover nucleotide distance thresholds were calculated for different taxonomic levels in order to test the error related to the use of fixed threshold for Chrysomelidae molecular identification. The new developed COI library includes nucleotide sequences of 261 Euro-Mediterranean Chrysomelidae species, 46 of them new to on-line repositories. The estimated barcoding efficiency (~94%) confirmed the usefulness of this tool for Chrysomelidae identification. The few cases of failure of the method were observed for closely related species, *e.g. Cryptocephalus marginellus* species complex, *Cryptocephalus violaceus – Cryptocephalus duplicatus* and some Altica species, where morphologically different species share the same COI haplotype. Different optimal thresholds were achieved for the tested taxonomic levels, confirming that a group-specific threshold significantly improves the accuracy of molecular identifications of Chrysomelidae.

Keywords: Chrysomelidae, DNA Barcoding

PO454

IS MONOPHAGY AN EVOLUTIONARY DEAD END? EVIDENCE FROM EURO-MEDITERRANEAN CHRYSOMELIDAE

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Chrysomelidae is a large family of phytophagous insects that include species with different levels of specialization and width in term of exploited host plants spectrum: monophagy, oligophagy and polyphagy. With this study, we would like to address the following biological question: Is the evolutionary pathway of host plant exploitation towards specialization? For this purpose i) the types of phytophagy (*i.e.*, monophagy, oligophagy and polyphagy) associated with 527 Euro-Mediterranean leaf beetles species will be defined using literature information and field observations on the host plants ii) a phylogeny of these species will be inferred using mitochondrial and nuclear markers (*i.e.*, COI, 16S rRNA, EF-1, 18S rRNA), sequences will be mined from public repositories and *de novo* amplified; iii) the types of phytophagy will be reconstructed across the leaf beetles phylogeny, in order to define if the evolutionary pathway is towards specialization (*i.e.* from polyphagy to monophagy) or monophagy occurred randomly. Up to now, a database including the information on the host plants has been developed and used to define the types of phytophagy associated with each analysed species. Moreover, a dataset of COI sequences belonging to the 527 Chrysomelidae species considered have been created (277 mined and 250 *de novo* sequenced); 30 EF-1, 204 16S rRNA and 207 18S rRNA sequences have been mined from online repositories. DNA extraction and PCRs for completing the datasets of the selected markers are currently ongoing. The study will improve the knowledge on distribution and evolution of the types of phytophagy in Chrysomelidae and, in addition, it will be useful for understanding the evolutionary pathway of trophic specializations in phytophagous insects.

Keywords: Chrysomelidae, host plants, phytophagy

PO455

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *LEMA BILINEATA*, A NEW INVASIVE SPECIES FOR EUROPE

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During the summer of 2017, the Tobacco Slug Beetle *Lema bilineata* (Germar) (Coleoptera; Chrysomelidae: Criocerinae) was detected on *Physalis peruviana* L., and later on, on *Salpichroa origanifolia* (Lam.) Thell., in the province of Naples (Italy). This is the first record of this species for Europe. Even though *L. bilineata* is reported to feed on numerous species of Solanaceae, its only host of economic importance is *Nicotiana tabacum* L. On this crop this chrysomelid is considered a pest in its native area, the South America, as well as in South Africa, where it widespread probably during the Boer War. Successively the pest was accidentally introduced to Australia. In the latter country, where tobacco growing is illegal, *L. bilineata* was considered a potential control agent of weeds. Despite its potential as economic pest, the majority of available data on *L. bilineata* are relative to its feeding preferences, while morphological, anatomical and molecular data are lacking. We collected data on chromatic pattern of *L. bilineata*, which shows stability of the elytrae, but a wide variability of the pronotum and the ventral region. At microscopic comparisons phallobase profile is similar to those of *Lema trilinea* White and *Lema trivittata* Say, but the internal sac is slightly different. Spermatheca is moderately convoluted as in other Lema species. Italian and South African specimens of *L. bilineata* were sequenced for COI, and phylogenetic analyses, including available geneBank Lema sequences, recovered *L. trilinea* and *L. trivittata* as its closest related species. L. trilinea has been recently introduced to South Africa, causing additional damage to tobacco crop. These information provide adequate tools to discriminate among related species at different developmental stages, thus contributing to prevent further introduction of invasive species.

Keywords: Barcoding, Chromatic Pattern, Chrysomelidae, Phallobase, Phylogenesis, Spermatheca, Tobacco

PO456

PHYLOGEOGRAPHY OF *TRICHOSIROCALUS HORRIDUS* (PANZER, 1801) (COLEOPTERA: CURCULIONIDAE, CEUTORHYNCHINAE) IN SOUTH-WESTERN EUROPE

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The genus *Trichosirocalus* includes 16 species with a Palaeartic distribution that mainly feed on Plantaginaceae and Asteraceae. In particular *Trichosirocalus horridus* shows a distribution from Iberian Peninsula to Caucasus and an association to vegetal species belonging to the genera Carduus, Cirsium and occasionally Onopordum. Because climate changes, particularly during the Quaternary climatic oscillations, have long been recognized to be important conditions for demographic history, population structure and intraspecific diversity in many taxa in Europe, we investigated the phylogeographical pattern in Italian, French, Spanish, German and Turkish populations of *T. horridus*, using a genetic mitochondrial marker (COI gene). A median-joining network shows 77 unique haplotypes where two major haplogroups are highlighted, one exclusive for the Italian populations and one that shows French and Spanish populations clumped within the same cluster, revealing a clear-cut phylogenetic clustering and geographical pattern. Mismatch distribution and neutrality tests provide evidence of a demographic expansion dating back to Pleistocene glacial phases. The time since expansion estimated for the Italian populations is nearly 200 Kya, that is datable to the Riss glacial period while for the Spanish-French populations is nearly 50 Kya, datable to the Würm glacial period. Accordingly, we can hypothesize a contraction of the populations of *T. horridus* in the southern refugial areas (Italy and Spain) during the glacial periods with a subsequent expansion towards the northern areas during the interglacial periods. Nevertheless, the isolation time was not enough to allow a strong genetic differentiation. Phylogeographic pattern of *T. horridus* showed the impact of Quaternary glaciations in shaping the genetic structure of its populations.

Keywords: *Trichosirocalus horridus*, Coleoptera, phylogeography, Quaternary glaciations, glacial refugia, south-western Europe, mtDNA

PO457

PHYLOGENETIC STATUS OF *PIMPLA TURIONELLA* L. (HYMENOPTERA: ICHNEUMONINAE) BASED ON CYTOCROME OXIDASE I

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Pimpla turionellae L. (Hymenoptera: Ichneumonidae) is a poliphagous endoparasitoid that spends its immature stages in pupae of various lepidopterous species. It serves as a potential biological control agent of many lepidopterous pests. Phylogenetic analyses of *P. turionellae* were performed with samples collected from Turkey and BOLD Taxonomy. In this study, phylogenetic analysis was carried out at species and genus levels of *P. turionellae*. The genomic DNA was extracted from wasps' mitochondria-rich body tissue using Qiagen Dneasy kit. The obtained DNA was used as template for PCR amplification by using primers LC01490 and HC02198. PCR products were sequenced by ABI 3730XL Sanger Sequencer (Macrogen, Holland). The COI sequences of Subfamily Ichneumoninae from BOLD taxonomy browser and P.turionellae from Turkey were used for analyses. Haplotype analysis of the COI sequences of *P. turionellae* was performed on the DnaSPv6.10.04 software. All COI sequences were aligned by Clustal W. Evolutionary divergence between species were estimated by pair wise distance. Minimum Evolution (ME), Maximum Parsimony (MP), Maksimum Likelihood (ML), and Neigbor-Joining (NJ) trees were conducted at subfamily, genus, and species levels in MEGA7. In the present study, all genera of the Ichneumoninae subfamily were distributed similarly at ME, NJ and ML analyses. Genera Pimpla, Tromatobia, Clistopyga, Endromopoda, Ephialtes, and Liotryphon were clustered. As a result of ML, NJ, and ME trees, *P. turionellae* species was proven to be closest to *Pimpla pedalis*, *P. croceipes*, *P. molesta*, *P. aquilonia*, *P. spuria*, and *P. sodalis* clustered in the same main branch with *P. turionellae*. In this haplotype analysis, TR-PTHap1-Turkey was the closest haplotype to CAN-PTHap4-Canada, KR791223. Our results would contribute to the biodiversity and solution of controversies in phylogenetic relationships of *P. turionellae*.

Keywords: *Pimpla turionellae*, COI, Phylogeny, Turkey

PO458

GENETIC VARIATION IN THE INVASIVE POTATO BEETLE POPULATIONS IN TURKEY BASED ON NUCLEAR (ACHE2 GENE) AND MITOCHONDRIAL DNA (CYTOCHROME OXIDASE I AND II SUBUNITS) MARKERS

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Colorado potato beetle, *Leptinotarsa decemlineata*, Say (Coleoptera: Chrysomelidae), is a serious insect pest of potatoes and other Solanaceae crops. It is known that the distribution through out the world is from North America to Europe and Asia. Potato beetle is commonly found in potato growing fields in all geographic regions in Turkey. But, genetic structure and genetic basis of insecticides-related differences of populations in Turkey

are unknown. This study focused on genetic differences in Colorado potato beetle populations in Turkey. To infer the genetic differences based on cytochrome oxidase I and II subunits (COI+II) and acetylcholinesterase 2 (AChE2) gene region associated with OP resistance in different beetle populations in Turkey, we analyzed sequences of 577 bp of the mtDNA gene region and sequences of a large fragment of 1037 bp polymorphically specified AChE2 region, respectively. Then, we combined and analyzed the data with the published sequences of potato beetle haplotypes that are available in the NCBI Genbank Database. Based on mtDNA gene region, common haplotype in Europe was found in potato beetle populations in Turkey. The sequences of AChE2 region in different populations were also aligned with haplotypes from North America and Europe. The haplotypes of AChE2 region in the studied populations of the different regions in Turkey were commonly found across Europe. Consequently, finding common European haplotypes indicate that Europe is the possible source of the potato beetle populations in Turkey. This study was supported by TÜBİTAK (project number 114Z945)

Keywords: *Leptinotarsa decemlineata*, cytochrome oxidase I and II subunits, Acetylcholinesterase gene, genetic variation, Turkey

PO459

THRIPS OF CULTIVATED SOLANACEAE IN BISKRA REGION (ALGERIA)

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Solanaceae are economically important crops in Biskra region and are attacked by several pests, among them thrips, but these are not identified and known in this region. In this study thrips were inventoried on different crops of the Solanaceae family (tomato, pepper, pepper and eggplant), during the 2009/2017, in agricultural exploitations, using shaking method, or direct collection of thrips from plants. The study identified 16 species of thrips including: *Odontothrips loti*, *Thrips minutissimus*, *Thrips tabaci*, *Aeolothrips intermedius*, *Melanthrips fuscus*,... etc. A. intermedius thrips is a facultatifpredator. Some of these insects can cause significant damage, as they can transmit dangerous viral diseases including tospovirus, example species *Frankliniella occidontalis*, *F. intosa*, *Thrips tabaci* and *T. falvus*.

Keywords: Thrips, species, solanaceae, Biskra

PO460

HOW TIGHT ARE BEETLE HUGS?

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Sexual selection and mating behaviour in insects are fascinating phenomena, challenging experimental studies of the dynamic interactions. Similar to other leaf beetles, rosemary beetles *Chrysolina americana* exhibit a distinct sexual dimorphism in tarsal attachment setae. Setal discoid terminals occur only in males, and they have been previously associated with a long-term attachment to the female's back (elytra) during copulation and mate guarding. For the first time, we studied living males and females holding to female's elytra. Pull-off force measurements with a custom-made tribometer featuring a self-aligning sample holder confirmed stronger attachment to female elytra compared with glass in both males and females; corresponding to 45 and 30 times the body weight, respectively. In line with previous studies, males generated significantly higher forces than females on convex elytra and flat glass, 1.2 times and 6.8 times, respectively. Convex substrates like elytra seem to improve the attachment ability of rosemary beetles, because they can hold more strongly due to favourable shear angles of legs, tarsi and adhesive setae. A self-aligning sample holder is found to be suitable for running force measurement tests with living biological samples.

Keywords: Biomechanics, Chrysomelidae, copulation, force experiment, functional morphology, mating, sexual selection, tarsus

PO461

Poster moved.

Session 12.
Parasitology, Pathology and Immunity

Immune Defences and Virulence Strategies

PO462

CHARACTERIZATION OF TWO NOVEL LECTINS IN THE INNATE IMMUNITY FROM *TRIBOLIUM CASTANEUM*

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Lectins are proteins that bind to specific carbohydrate structures and can thus recognize particular glycoconjugates of cell membranes using structurally related Ca2+-dependent carbohydrate-recognition domains (CRDs). Most animal lectins can be classified into four distinct families: C-type lectins; P-type lectins; pentraxins; and galectins. Currently, *Tribolium castaneum* latrophilin (TcLPH), a G protein-coupled receptors, contains galactoside-binding lectins (galectins) in its extracellular domain. This suggests that TcLPH may be involved in the immune response in *T. castaneum*. Meanwhile, a C-type lectin are identified from *T. castaneum*, designated as TcCTL. Further analysis is found that these two lectins contained one CRD, indicating that the TcLPH and TcCTL may have a similar immune function in *T. castaneum*. In this study, the expression of TcLPH and TcCTL are significantly regulated after lipopolysaccharide (LPS), peptidoglycan (PGN), *Escherchia coli* or *Staphylococcus aureus* stimulation, indicating that these lectins involve in the immune response to bacterial infection. Sugar binding assay suggest that the TcLPH and TcCTL can bind to the glycoconjugates of bacteria surface, such as LPS, PGN and they can compete with bacterial as competitors. Test results ulteriorly indicate that the recombinant TcLPH and TcCTL can agglutinate Gram-positive (*S. aureus*, *Bacillus subtilis* and *Bacillus thuringiensis*) and Gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*) in the presence of calcium and also can bind to these bacteria. In addition, knockdown of TcLPH and TcCTL by dsRNA *in vitro* result in the survival rate of *T. castaneum* is significantly decreased during *E. coli* and *S. aureus* infection, further indicating that TcLPH and TcCTL play an important immune role in *T. castaneum* to bacteria challenge. Collectively, TcLPH as well as TcCTL, containing a CRD, play roles in immune response towards pathogen infection through agglutinating the bacteria in the presence of calcium in *T. castaneum*.

Keywords: Galectin; C-type lectin; combine; agglutination; immunity; infecion

PO463

TRANSCRIPTOMIC ANALYSIS OF A NOVEL VIRAL SYMBIONT REVEALS DIFFERENTIAL EXPRESSION DURING VIRUS REPLICATION IN PARASITOID WASPS AND FLY HOSTS

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For parasitoid wasps, successful development as a parasite results in the death of the host insect. Host survival thus depends on the ability of its immune system to kill the invading wasp, while wasp survival depends on evasion of such immune responses. As a result, wasps and their hosts have coevolved strategies to gain an advantage in this evolutionary arms race. Although normally considered to be strict pathogens, some viruses have established persistent infections within parasitoid wasps and serve as symbionts to the wasps via host immune suppression during parasitism. Mutualistic associations between viruses and parasitoid wasps have evolved independently multiple times, but most of these systems remain largely understudied. Therefore, characterization of independent examples of this phenomenon is necessary for understanding the processes underlying the evolution of viral mutualism. The wasp species *Diachasmimorpha longicaudata* carries a symbiotic poxvirus that is injected into the tephritid fly hosts of the wasp during oviposition. Although this virus, known as *D. longicaudata* entomopoxvirus (DlEPV), has been observed inside the venom gland of female wasps and within hemocytes of parasitized hosts, an understanding of the biology and evolution of this association is currently lacking. We sequenced the DlEPV genome and utilized functional genomic tools to gain insight on how DlEPV can attack fly host cells during parasitism and conversely, replicate in wasp cells prior to eclosion without causing harm. We found that most DlEPV replication genes are significantly upregulated in wasps compared to in hosts, while most DlEPV putative virulence genes are significantly upregulated in hosts. These data therefore support a dichotomy of viral function, where virus replication is promoted in wasp tissue and virulence in host tissue. Such a division of viral activity could represent an important adaptation to maintain a stable symbiosis.

Keywords: Parasitoid, virus, symbiosis, genome, evolution

PO464

IMMUNE AND EPIGENETIC ASPECTS OF WAX MOTH *GALLERIA MELLONELLA* RESISTANCE TO *BACILLUS THURINGIENSIS* DURING EXPERIMENTAL EVOLUTION

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Bacillus thuringiensis (Bt) is a widespread Gram positive entomopathogenic bacterium that has been developed as a biopesticide to control pest and vector insects. There is increasing evidence for the occurrence of Bt-resistance among treated insects in the field. In order to explore the mechanisms behind Bt resistance in insects we used the larvae of the Greater wax moth *Galleria mellonella* which have been established as model hosts for bacterial infections. We experimentally selected a *G. mellonella* line over 30 generations for resistance against Bt. The latter exhibited a 11-fold increased resistance when compared with the non-selected line and was used to study immune and stress responses, and epigenetic mechanisms upon challenge with a Bt spore-crystal mixture. The resistant line exhibited differences in innate immune and stress responses as well as in the gut microbiome when compared to the susceptible line. Our results suggest that epigenetic mechanisms operating at the pre-transcriptional and post-transcriptional levels contribute to the transgenerational inherited transcriptional reprogramming of stress and immunity-related genes. Interestingly, our study elucidated trade-offs which will be discussed in the light of current insights into mechanisms behind Bt pathogenesis in *G. mellonella*.

This work was supported RSF №16-14-10067.

PO465

THE ROLE OF MIDGUT IMPI AND GLOVERIN IN THE RESISTANCE OF WAX MOTH *GALLERIA MELLONELLA* TO *BACILLUS THURINGIENSIS*

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Bacteria *Bacillus thuringiensis* (Bt) is one of the most common sources of biopesticide used for pest management. Many cases of insect's resistance to Bt toxins were registered last years. Greater wax moth *Galleria mellonella* is a popular insect model host to test different kind of pathogen. Previously we have shown that the wax moth immune system makes important contributions to insect inducible resistance to Bt. In this study the level of expression of antimicrobial peptides (AMP), IMPI and stress related genes in the fat body and midgut have been investigated during sublethal and half-lethal natural bacterial infection by Bt, in order to examine the role of the immune and stress response in protecting insects during intestinal infections. We have detected significant elevated level of expression of both Gloverin in the fat body and Gallerimycin in the midgut under half-lethal dose of Bt (LC50) as compare with sublethal (LC15) dose of Bt. Both sublethal and half-lethal doses of Bt leads to elevated level of expression of stress related genes in the fat body. Interestingly, we found the elevated level of IMPI expression in midgut under both doses of Bt as compare with uninfected insects, and it was on dose-depend manner. The RNA interference of IMPI and Gloverin genes resulted in increasing susceptibility of insects to Bt.

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PO466

MATERNAL TRANSFER OF IMMUNITY ACCORDING TO AGE AND REPRODUCTIVE EFFORT IN AN INSECT

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Since lifetime is limited, organisms should optimize their allocation of resources between different life-history traits to maximize fitness, including immunity and reproduction. Because the latter are costly traits, their relative value is likely to change during an organism's lifespan. In particular, levels of investment in immune defence that an individual should achieve to self or its progeny as a function of its age and reproductive effort are still unknown. A particular aspect of costly immune investment in invertebrates is trans-generational immune priming (TGIP), through which immune-challenged mothers transmit immune protection to their offspring. Such an immune priming allows improving the offspring performance if the maternal septic environment persists across generations. For example, bacterially immune-challenged females of the mealworm beetle, *Tenebrio molitor*, provide a variable number of their egg with antibacterial activity, at the expenses of the number of eggs they produce. Here, we examined how bacterially immune-challenged females of T. molitor are trading-off their investment in reproduction and TGIP according to age and past reproductive effort. Overall, we predict that reproductive effort and egg protection should decline with female age, and past reproduction may curtail egg protection.

Keywords: Immunity, aging, trans-generational immune priming, maternal effect, antibacterial activity

PO467

EDL/MAE, A MAJOR GENE FOR *DROSOPHILA MELANOGASTER* IMMUNE RESISTANCE TO THE PARASITOID WASP *LEPTOPILINA BOULARDI*

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Drosophila melanogaster is a main model in biology, notably immunity and evolution. Although its innate immune processes to fight bacteria and fungi have largely been explored, less is known of the defence against endoparasitoid wasps whose successful development inside the insect host leads to its death. One of the most studied Drosophila - parasitoid wasp interaction involves *Leptopilina boulardi* that lays eggs inside host larvae and develop at their expense. Once the parasitoid egg has been recognized as a foreign invader, the Drosophila larva can mount a successful immune response, the encapsulation: The egg is surrounded by several layers of hemocytes, the so-formed capsule being melanized, which kills the parasitoid. Alternatively, the immune response can be circumvented thanks to the venom components injected by the female wasp together with the egg. Using two Drosophila strains, resistant and susceptible to *L. boulardi*, which differ only in a region of chromosome 2R containing a major resistance gene, we previously identified edl/mae (R and S alleles) as a candidate gene. To decipher its possible role and identify the molecular and cellular events leading to success or failure of encapsulation, we used various approaches from fly genetics to flow cytometry. We confirm here the involvement of edl/mae in Drosophila resistance since overexpression of the resistant allele in a susceptible background leads to a resistant phenotype, and interference of the expression of the susceptible allele results in an increased rate of parasitoid encapsulation. At the cellular level, the peak of the number of hemocytes post-parasitism occurred earlier in the R strain, as did the bursting of the hematopoietic lymph gland. We are now exploring the pathways supporting the role of edl/mae in the kinetic hematopoietic response to parasitism, and more broadly in the encapsulation process leading to resistance.

Keywords: Drosophila, immunity, resistance, *Leptopilina boulardi*

PO468

A CONSTITUTIVELY EXPRESSED ANTIFUNGAL PEPTIDE PROTECTS *TENEBRIO MOLITOR* DURING A NATURAL INFECTION BY THE ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA*

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Inducible defenses are predicted to evolve when they are costly to express and would benefit the host organism only if they are deployed when needed, *i.e.* in the presence of a threat. The evolution of constitutive defenses on the other hand is supposed to be favored when the threat in the environment of the organism is constant. The insect immune system is well known for its use of a battery of antibacterial peptides, most of them being inducible

following infection. However, few antibacterial peptides are constitutively expressed. This is the case of Tenecin 3, an antifungal peptide of the mealworm beetle *Tenebrio molitor*. In this study, we use a gene knock-down approach to explore the role of this constitutive defense during a natural infection of T. molitor with the entomopathogenic fungus *Beauveria bassiana*. We discuss the changes in the fitness of the host in regard to the progression of the infection, as well as the impact of the down-regulation of an immune effector on the trajectory of the infection in the host.

Keywords: *Beauveria bassiana*, *Tenebrio molitor*, antifungal peptide, immunoecology

PO469

ENTOMOPATHOGENIC BACTERIA STRUGGLE TO OVERCOME INSECT IMMUNE DEFENCES. THE CASE OF *BREVIBACILLUS LATEROSPORUS*

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Bacteria have coevolved with insects developing varying level of interaction, including beneficial and pathogenic relationships. Entomopathogenic bacteria developed several tools and strategies to overcome host’s defence mechanisms and reach insect body sections where to develop and subsequently spread in the environment. *Brevibacillus laterosporus* is a bacterium characterized by a broad spectrum activity against invertebrates, like insects, nematodes, and molluscs. Recent genome sequencing and annotation of highly pathogenic strains of this microorganism revealed a variety of toxins and virulence factors implied in pathogenesis, which helps to understand how this species establishes advantageous relationships with insects, leveraging an efficient and complex machine at its own service. These aspects are discussed in relation to the results of our recent studies leading to the identification of enzymes, toxins, and other virulence factors, including proteases, chitinases, spore surface proteins, protective antigen proteins, antibiotics, and new insecticidal crystal (Cry) toxins. The expression of these target genes was studied either *in vitro* and *in vivo* at the transcriptional level, highlighting their role within the insect body environment during pathogenesis. On the other side, we investigated the insect immune defence mechanisms activated against this pathogen in the insect host midgut, that represent the primary site of action. Employing a house fly model, the activation rate of immune related genes in the midgut tissues, was assessed in insects challenged with *B. laterosporus*. As a result, several antimicrobial peptides (AMPs) and enzymes (*i.e.*, prophenoloxydase) were found to play a key role in counteracting the pathogenic action of the bacterium at the intestinal level. Enhancement of bacterial virulence was therefore achieved by fly immune-impairment employing an immunosuppressive agent. These results support the development of immune-impairing tools to improve the efficacy of entomopathogens in integrated pest management programs.
This study was supported by MIUR (PRIN project 2015 “BIOPIC”, 2015BABFCF).

Keywords: AMP, prophenoloxydase, virulence factors, Cry toxins, midgut

PO470

GENERATION AND ANALYSIS OF A COMPREHENSIVE *TOXONEURON NIGRICEPS* TERATOCYTE TRANSCRIPTOME

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Insect host-parasitoid systems are examples of fascinating and complex interactions. Parasitoids have adapted to their respective hosts by developing several strategies ensuring the success of parasitism, providing physiological alterations and overcoming the immune response of their host. Maternal factors (venom, calyx fluid with or without polydnaviruses, and ovarian proteins), introduced into the host by the female wasps during egg deposition together with embryonic factor (teratocytes), play a key role in host regulation and immune suppression. Teratocytes are specialized cells deriving from the dissociation of the membrane surrounding the parasitoid embryo(serosa), and are released in the host haemocoel when the parasitoid egg hatches. They release several molecules impacting physiology, development and nutritional suitability of the host. To date only a single protein (a putative chitinase) has been identified in *T. nigriceps* teratocytes. Here, we use high-throughput RNA sequencing (RNA-Seq) to generate a *de novo* transcriptome of *T. nigriceps* teratocytes. RNA-Seq data assembly resulted in 24,569 contigs which were subsequently annotated using the BLASTx algorithm against the NR database and further functionally analysed using the Blast2GO software. The distribution of the species associated with top BLAST hits shows that a high proportion of genes in the *T. nigriceps* teratocyte transcriptome is most similar to genes belonging to different parasitoid wasp species, in particular, to Microplitis demolitor. Among the predicted proteins identified, several are potentially involved in host immunosuppression such as ovalbumin-related X-like proteins, serpin proteins, chitinases and Rho GAP proteins. Moreover, similar to the transcriptomic analysis of *M. demolitor* teratocytes, we identified putative antimicrobial peptides. These preliminary results led us to hypothesize that teratocytes are not only responsible for host immunosuppression but at the same time could prevent host infection by pathogen intruders, supporting the immunosuppressed host through the production of parasitoid-derived AMPs.

Keywords: *Toxoneuron nigriceps*, Teratocytes, transcriptomic analysis, host immunosuppression

PO471

MUTUAL PROTECTION OF ENDOPARASITOIDS BY SYMBIOTIC BRACOVIRUSES

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Bracoviruses are large double-stranded DNA viruses with their genomes stably integrated into the genomes of their associated parasitoid wasps. The virus elements replicate in the female wasps, assemble into virions containing DNA that encodes virulence genes. Virions are injected into the host during wasp oviposition, the virus particles infect different host cell types but do not replicate. The wasps use the virus to ensure the survival of their offspring in the host through modifications in host physiology, neuroendocrine and immune responses. We investigated the specificity of two bracoviruses with their associated wasps, *Glyptapanteles lipardis* and *G. porthetriae*, in larvae of the gypsy moth, *Lymantria dispar*. To separate parasitoid eggs and virions, we used gamma-irradiated, sterilized wasps that inject intact virus particles at oviposition, but non-viable eggs. The host having received the virions of one wasp species was then used as recipient of viable, virion-free eggs of the other wasp. These parasitoid eggs were recovered from normally parasitized hosts. This procedure was performed reciprocally with both wasps and their associated viruses. Hosts with injected virions and implanted parasitoid eggs were dissected periodically to assess the stage of the parasitoid. Virions of the gregarious wasp *G.*

liparidis supported the development of the solitary *G. porthetriae* parasitoids, but the final instar parasitoids did not emerge from the host. The reverse experiment produced almost the same results. When virions and eggs of the same species were combined in the host, the parasitoids developed to fully-grown larvae that emerged (*G. liparidis*) or did not emerge (*G. porthetriae*) from the host. Results from the cross-protection experiments suggest that the virions of one wasp species protect the egg of the other species from encapsulation by host hemocytes to allow parasitoid survival and development to maturity, however they do not promote emergence of the parasitoids from the host.

Keywords: Polydnaviruses, parasitic wasps, immune protecion, Glyptapanteles, gypsy moth

PO472

LINKING GENETIC VARIATION IN DISEASE RESISTANCE AND TOLERANCE WITH HETEROGENEITY IN DISEASE SPREAD

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Host heterogeneity in disease transmission is widespread and presents a major hurdle to predicting and minimising the spread of infectious agents. The causes of this variation and how it scales up to population-level outcomes is however, poorly understood. Our lab takes an experimental approach to this problem, using *Drosophila melanogaster* and its viral pathogen Drosophila C Virus (DCV) as a model system for experimental epidemiology. Here, we sought to understand the link between genetic variation in the ability to resist and tolerate viral infection with heterogeneity in key components of transmission. Flies exposed systemically to a range of DCV concentrations show extensive genetic variation in host vigour and in the sensitivity to increasing pathogen concentrations, two key components of disease tolerance. We also find pervasive and complex effects of genetic background, sex and mating status on key determinants of viral transmission: social aggregation, viral load at death, initial viral growth and viral shedding. We then use this data – which offers a level of empirical control and individual-level detail that is rare in most wild or domesticated animal systems – to parameterise an epidemiological model to ask how individual-level heterogeneity in these traits affect heterogeneity in epidemic dynamics.

Keywords: Infection, pathogen transmission, host heterogeneity, resistance, disease tolerance, Drosophila C Virus

PO473

INNATE PARASITOID RESISTANCE IN THE POTATO APHID, *MACROSIPHUM EUPHORBIAE*

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Macrosiphium euphorbiae is an economically important pest, vectoring over 50 plant viruses throughout major crops such as tomato, strawberry, raspberry and potato. Integrated pest management (IPM) systems for *M. euphorbiae* could be developed to make use of natural enemies of the aphid, such as parasitoid wasps. Previous research has identified eight genotypes within this aphid species, one of which exhibits innate resistance to the generalist parasitoid *Aphidius ervi*; this finding contrasts with some other aphid species, where parasitism-resistance can be conferred by facultative bacterial endosymbionts. Through genomic studies, we aim to clarify the mechanism of aphid-encoded innate resistance, as well as understand the frequency of the resistant population in the wild. Aphids have been sampled from commercial fields and garden sites in the Summer of 2016 and 2017 with aphid genotype and parasitoid susceptibility assessed. Surprisingly, we see resistant populations at a low level in comparison with susceptible genotypes, suggesting an undetermined cost associated with innate resistance. The genome of seven lines of *M. euphorbiae* belonging to the low parasitoid susceptibility genotype was sequenced with the Illumina HiSeq and one line further sequenced using the PacBio Sequel platform. Analysis of sequence data was conducted to check for the presence of cryptic endosymbionts that might confer resistance. However, there was no evidence for the presence of such cryptic symbiont, providing additional evidence that parasitoid resistance is the likely result of innate immunity. In addition, genome sequencing was conducted for 12 aphids lines belonging to five susceptible aphid genotypes. We will use the data for assessing structural variation and variant calling through read alignment of susceptible *M. euphorbiae* data to our previously generated PacBio reference genome data for the resistant genotype. From this, we can begin to discover what genes are involved in the process of parasitoid resistance.

Insect Gut Immunity and Microbiome

PO474

ANTIBACTERIAL ACTIVITY OF BACTERIA ISOLATED FROM THE GASTROINTESTINAL TRACT OF *MELOLONTHA* SPP. LARVAE

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Entomopathogenic nematodes (EPNs) from the families Steinernematidae and Heterorhabditidae (Nematoda: Rhabditida) are effective biocontrol agents against soil-dwelling stages of many insect pests. They are symbiotically associated with entomopathogenic bacteria (EPB) of the genera *Xenorhabdus* and *Photorhabdus*. A significant progress in applying these bioagents to reduce the populations of pests belonging to Scarabaeidae (Insecta: Coleoptera) is observed last years. EPNs are widespread almost all over the world. The only free living developmental stage are infective juveniles. Nematodes infect insects through natural body openings and release symbionts in host hemocel. Bacteria multiply quickly in the hemolymph causing insect death. A growing bacterial population provide an optimal environment for nematodes by decomposing host tissues and inhibiting the growth of other bacteria.

Up till now, researchers have only found evidence for unilateral relations resulting from the fact that EPB produce a number of substances that inhibit the growth of insect gut bacteria. There have been no studies on antagonistic mechanisms that would work in the opposite direction, *i.e.* production of substances that inhibit the growth of EPB by bacteria of the insect gut microflora. The goal of the study was to isolate and identify bacteria from the midgut of *Melolontha* spp. larvae, which would be capable of inhibiting the growth of *Xenorhabdus* spp. and *Photorhabdus* spp. A total number of 900 bacterial strains were isolated from the midgut of 60 individual *Melolontha* spp. larvae. Antibacterial activity was determined using several types of growth inhibition plate assays. One half of the isolates were tested under aerobic conditions and the other half under microaerophilic conditions. 37 isolates showed antibacterial activity against EPB. Selected strains were identified by molecular methods. The growth of *Xenorhabdus* spp. and *Photorhabdus* spp. was most strongly inhibited by isolates identified as members of the genera *Pseudomonas*, *Serratia*, *Acitenobacter* and *Citrobacter*.

Keywords: Bacterial interactions, midgut, *Melolontha*, *Xenorhabdus*, *Photorhabdus*

Host Regulation by Parasites and Pathogens

PO475

NUTRITIONAL EFFECTS ON HOST DEFENCE INDUCED BY *SERRATIA MARCESCENS* IN BATROCERA TRYONI

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Nutrition is closely linked to host immune defence against pathogens. However, how nutrition and resistance to infection interact remains to be fully understood. Here, we aim to provide an insight into the effects of nutrition on the host resistance after infection. We systemically infected female Q-flies with the bacterium *Serratia marcescens* and measured macronutrient intake and bacterial number. The result showed that, compared to naïve and sham-injected flies, flies infected with bacteria shifted their diet to a carbohydrate-biased food. We further found that higher-carbohydrate diet improved survival of infected host while having no effect on pathogen load. These results suggested an important role of dietary carbohydrate in host survival after bacterial infection. Because we have not been able to detect any decrease in the bacterial load of infected individuals, we suggested that diet promotes survival through tolerance mechanism.

Keywords: *Bactrocera tryoni*, bacterial infection, nutrition, *Serratia marcescens*, tolerance mechanism

PO476

BIOLOGICAL ROLE OF PLL AND PHL – LECTINS PRODUCED BY ENTOMOPATHOGENIC BACTERIA *PHOTORHABDUS LUMINESCENS* AND *PHOTORHABDUS ASYMBIOTICA*

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Photorhabdus is a genus of gram-negative bacteria which is known for its symbiotic relationship with entomopathogenic nematodes of the genus *Heterorhabditis*. Both bacteria and nematodes produce variety of toxins, proteases and other compounds which make them highly pathogenic for a wide range of insects. In our work we focused on lectins produced by bacteria *P. luminescens* and *P. asymbiotica*. Both species are known as highly virulent entomopathogens, but the latter is also able to cause serious and difficultly treatable infections in humans. Recently, we have identified two sequentially similar lectins designated as PLL and PHL in the genome of *P. luminescens* and *P. asymbiotica*, respectively. Recombinant forms of these lectins share high affinity towards L-fucose and both bind to the surface of red blood cells and haemocytes of *Galleria mellonella*. PLL agglutinates haemocytes and red blood cells A, whereas only red blood cells O were effectively agglutinated by PHL. Although we did not observe any damage on cells treated with lectins, neither the lectins were toxic to *G. mellonella* upon injection, PHL was found to be interfering with humoral immunity in both insect and human. PHL is recognised by immune system which reacts by increased melanisation in insects and increased production of reactive oxygen species in human blood observed *in vitro*. Interestingly, PHL inhibited production of reactive oxygen species elicited by neutrophil activators and decreased antimicrobial activity both in human blood and insect haemolymph. Despite many similarities, we show that the role of PLL and PHL is distinct. It is important to note that besides the involvement of studied lectins in infection and suppression of host immune system, they could also play role in symbiosis with nematodes or mediate interactions within bacterial population.
This work was supported by grants no. 17-03253S and 18-18964S from the Czech Science Foundation.

Keywords: *Photorhabdus asymbiotica*, *Photorhabdus luminescens*, lectin, innate immunity, agglutination, reactive oxygen species, antimicrobial activity, melanisation

PO477

Poster withdrawn.

PO478

HORIZONTAL TRANSMISSION OF A MICROSPORIDIUM IS ENHANCED BY RAIN

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The gypsy moth (*Lymantria dispar*), a lepidopteran species native to Europe and accidentally introduced to North America, is a serious pest of deciduous forests and fruit trees. Repeated defoliation of trees during mass outbreaks causes both ecologic and economic damage.Microsporidia are considered as important natural enemies and several species isolated from gypsy moth populations have been released in North America as classical biological control agents. Specifically, infections with *Nosema lymantriae* are assumed to contribute to in the collapse of *L. dispar* outbreak populations in Europe. Horizontal transmission of the infective spores to uninfected host larvae occurs predominantly via contaminated feces. Here, we tested the effects of simulated rain (irrigation) on spore transmission via feces in a two-story cage system that provided random distribution of spore-laden feces on oak or hornbeam foliage. After a 24 hour contamination phase, infected larvae were removed from the cages and groups of uninfected larvae were left to feed on the foliage for 72 hours to test transmission. Direct contact between the infected and uninfected larvae was avoided to prevent transmission pathways other than via feces. To test effects of disease progression on transmission efficiency, we used experimentally infected larvae in their early, middle, and late stages of disease progression. Transmission success increased significantly with disease progress and with simulated rain. For example, infection rates ranged from 4 to 17% when test larvae were exposed to feces from larvae in the middle stage of infection, but increased to 30 to 57% when leaf surfaces were wet. In summary, rain enhanced the transmission of *N. lymantriae* spores to gypsy moth larvae by increasing the number of frass pellets sticking to the leaves, dissolving the spores in the water droplets and thus, promoting the chance of being ingested by the feeding larvae.

PO479

THE EXPRESSION OF *TOXONEURON NIGRICEPS BRACOVIRUS* (TNBV) GENES INDUCES THE INHIBITION OF TOR PATHWAY GENE TRANSCRIPTION IN HELIOTHIS VIRESCENS PROTHORACIC GLANDS

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Polydnaviruses are obligate viral symbionts of Ichneumonid and Braconid wasps, which exclusively attack the larval stages of their lepidopteran hosts. These viruses are injected by females during oviposition and selectively infect several host tissues, through the expression of viral genes without undergoing any replication events, which eventually results in host physiology manipulation, altering the neuroendocrine balance. *Toxoneuron nigriceps bracovirus* (TnBV) is associated to *Toxoneuron nigriceps* (Hymenoptera: Braconidae), the endoparasitoid wasp of *Heliothis virescens* (Lepidoptera: Noctuidae). Previous studies showed that *H. virescens* parasitized by *T. nigriceps* has a normal development up to the last larval instar, but fail to pupate, due to the arrest of ecdysteroidogenesis. Indeed, the main target tissues of TnBV gene expression are the prothoracic glands (PGs), responsible for the production and secretion of ecdysone, the molting hormone, following the prothoracicotropic hormone (PTTH) stimulus. TnBV induces a functional inactivation of PGs, making them not responsive to the PTTH stimulus, resulting in a considerable decrease of ecdysone levels. In *H. virescens* we previously investigated the involvement of PI3K/Akt/TOR pathway in ecdysteroidogenesis. Here, we showed that this cellular signaling is one of the targets of TnBV infection. Results of western blot and enzyme immunoassay demonstrated that parasitization inhibits the ecdysteroidogenesis and the phosphorylation of two targets of TOR (4E-BP and S6K), despite the stimulation of PTTH contained in the brain extract. Moreover, using a transcriptomic approach, we identified the viral genes selectively expressed in PGs of parasitized last instar larvae and observed lower expression levels of genes involved in PI3K/Akt/TOR pathway. The down-regulation of several genes of the TOR pathway (tor, 4e-bp and s6k) in PGs of parasitized larvae was confirmed by qRT-PCR, supporting the hypothesis of TnBV involvement in blocking ecdysteroidogenesis, at least in part through the alteration of the TOR pathway at the transcriptional level.

Keywords: *Toxoneuron nigriceps bracovirus*, transcriptomic analysis, TOR pathway, prothoracic glands, gene downregulation

PO480

APHIDIUS ERVI TERATOCYTES RELEASE ENOLASE (AE-ENO) AND FATTY ACID BINDING PROTEIN (AE-FABP) BY EXOSOMAL VESICLES

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The molecular bases of the host-parasitoid interactions in the biological system *Acyrthosiphon pisum* (Harris) (Homoptera, Aphididae) - *Aphidius ervi* (Haliday) (Hymenoptera, Braconidae) have been elucidated allowing the identification of maternal (a GGT contained in venom secretion) and embryonic parasitic factors (Teratocytes) that regulate the host physiology after parasitization. Teratocytes, cells deriving from the dissociation of the embryonic serosa of the parasitoid, are responsible for an extra-oral digestion of the host tissues in order to provide a suitable nutritional environment for the parasitoid larva development. Teratocytes rapidly grow in size without undergoing any cell division, synthesize, and release in the host hemolymph two proteins: a Fatty Acid Binding Protein (Ae-FABP) involved in transport of fatty acids deriving from host tissues, towards the parasitoid larva, and an Enolase (Ae-ENO). Ae-ENO, is a glycolytic enzyme that, as extracellular protein, works as a plasminogen like receptor inducing its

activation in plasmin. Both Ae-FABP and Ae-ENO are released in extracellular environment although their amino acid sequences lack of the signal peptide. Here we investigated the mechanism by which teratocytes release Ae-FABP and Ae-ENO. Our results, obtained by western blot analyses and immunogold staining, demonstrate that these two proteins are localized in vesicles released *in vitro* by teratocytes. Moreover, immunofluorescence and immunogold assays using an antibody against the protein ALIX (ALG-2 interacting protein X,) confirm the exosomal nature of these vescicles. Indeed immunogold staining clearly show that Alix is localized in the cytoplasm of the teratocytes, in their membrane blebs and into the vesicles released from them. Alix together with other proteins such as TSG101, HSP70 and the tetraspanins CD63, CD81 and CD9 are considered and for this reason commonly used as a marker to identify and discriminate exosomes among different types of extracellular vesicles.

Keywords: *Aphidius ervi*, teratocytes, exosomes, Alix

PO481

FUNCTIONAL ANALYSES OF A γ–GLUTAMYL TRANSPEPTIDASE COMPONENT OF THE VENOM OF THE PARASITOID WASP *APHIDIUS ERVI* BY HETEROLOGOUS EXPRESSION IN *DROSOPHILA MELANOGASTER*

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Gamma glutamyl transpeptidases (GGTs) are key enzymes in the metabolism of glutathione (GSH), a major cellular antioxidant. Their involvement in multiple aspects of human physiopathology fostered intense research in mammalian systems, but very limited information is available in insects. A soluble GGT isoform (AeGGT1) was identified in the venom that the parasitoid wasp *Aphidius ervi* injects at oviposition into the body of the aphid *Acyrthosiphon pisum*. By triggering apoptosis in host ovaries, it exerts a major impact on host reproduction. To shed light on the molecular mechanisms underlying AeGGT1 harmful effects, we generated *Drosophila melanogaster* transgenic lines conditionally expressing a soluble or a membrane-anchored AeGGT1 recombinant protein. RT-PCR, western blot and immunofluorescence experiments confirmed that both genes were transcribed and both proteins were produced and correctly secreted/localized in the heterologous system. Phenotypic analyses did not show any significant effect of AeGGT1 on fly viability or fertility. However, flies overexpressing AeGGT1 were more tolerant to hydrogen peroxide oral administration than isogenic control flies. Interestingly, GGT activity was significantly reduced in AeGGT1 transgenic flies, which also showed decreased cysteine levels. Finally, down-regulation of a *Drosophila melanogaster* endogenous GGT gene (DmGGT1) also resulted in increased resistance to oxidative stress. Taken together, these results suggest that AeGGT1 may inhibit the activity of DmGGT1 leading to extracellular accumulation of GSH, which, in turn, may protect from oxidative stress. The implications of our data with respect to the action of AeGGT1 in parasitized aphids will be discussed.

Keywords: GGT, *Aphidius ervi*, *Drosophila melanogaster*, parasitoid venom, heterologous expression

PO482

GALLERIA MELLONELLA AS A MODEL HOST FOR MICROSPORIDIA

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The greater wax moth *Galleria mellonella* is a widespread economically important pest of apiculture, as well as a popular laboratory model. Several microsporidia are known to infect *G. mellonella*. A specific pathogen *Nosema galleriae* was described in 1977 by J.J. Lipa. This description was based solely upon light microscopic observation and thus cannot be validated in the view of modern systematics using electron microscopy and molecular genetic data. A microsporidium *Vairimorpha ephestiae* from the Mediterranean flour moth *Ephestia kuehniella* was successfully transmitted to *G. mellonella* under lab conditions by J. Weiser and this model was further exploited for cytological and biochemical studies of parasite-host interactions (Lozinskaya *et al*., 2004; Vorontsova *et al*., 2004, 2006). Intrahaemocoelic injections were successful for spores and prespore developmental stages of *Vairimorpha (Nosema) plodiae* and *Vairimorpha (Nosema) heterosporum* from the Indian meal moth *Plodia interpunctella* (Weiser, 1975). Recently, two specific pathogens of honey bees, *Nosema apis* and *Nosema ceranae*, were revealed in *G. mellonella* collected from the bee hives (Ozgor *et al.*, 2017), thus expanding our knowledge of host range of these microsporidia to Lepidoptera. A microsporidium of the genus *Tubulinosema* isolated from beet webworm *Loxostege sticticalis* showed high infestation rate when transmitted to *G. mellonella*, even after storage in a dry host cadaver over 12 months. Conversely, a specific parasite of European corn borer, Nosema pyrausta showed mean infestation rate of 0 and 5% in *G. mellonella* (3 million spores per II instar larva) at 30 and 24 deg C, respectively. Taken together, these findings indicate that the greater wax moth can be used as a laboratory model with varied level of susceptibility to microsporidia of different species.

The research is supported by Russian Science Foundation, grant # 16-14-00005.

Keywords: Galleria, Microsporidia, Nosema, Vairimorpha, *Tubulinosema*, host range

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Physiology and Biochemistry

Insect Development

PO483

ESTABLISHMENT OF AN EFFECTIVE AND FAST ASSAY TO DISTINGUISH MALE AND FEMALE EMBRYOS OF *BACTROCERA DORSALIS*

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The oriental fruit fly, *Bactrocera dorsalis* (Hendel), is a serious agricultural pest in many Asian countries. A Y-linked male determiner (M-factor) has been hypotheses that involving in male embryonic development of the pest to inhibit normal development of female, but it has not been identified yet. Comparison the different expression patterns between male and female at the early developmental stage give an ideal way to identify the male-specific factor(s). Science enough starting material is needed to many future studies, a fast and easy assay for embryonic sexing was established in the study. In the first part, a candidate of male-specific DNA marker was tested to confirm its sex-specificity. Genomic DNA from heads of individual male and female adults were isolated, two nest PCR amplifications with specific primers were conducted. The single PCR products, 325 bp, only be detected in male samples as expected. In the following experiments, total 72 embryos were collected within two-hour after egg laying, and homogenized in 10 μL Tris buffer individually. The 5% extract was used for sex distinguish assay and the rest 95% was kept in deep freezer for future RNA isolation. In our protocol, the first PCR was perform using regular thermocycler; then we diluted the first PCR products as templates for the second nest PCR using SYBR Green real-time system. From the vast differences of Ct values, we can easily to distinguish the 33 male and 39 female embryos. This method is effective, easy to operate, and it can be performed within a single day. This novel assay is particular useful to identified sex-specific regulators that has potential to apply in SIT control for *B. dorsalis*.

Keywords: Male determiner (M-factor), *Bactrocera dorsalis* (Hendel), embryonic sexing

PO484

EARLY EMBRYONIC DEVELOPMENT IN *BEMISIA TABACI*

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The silverleaf whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae), is a highly invasive and destructive agricultural pest, with a global distribution. As a vector of plant viruses and by excreting honeydew which attracts mould, *B. tabaci* causes substantial economic losses to growers of more than 600 different plant species. Options for *B. tabaci* control are limited as this insect has evolved various insecticide resistance mechanisms. New management tools would offer growers valuable alternative options to protect crops. To enable discovery of new control methods, more knowledge on the fundamental biology of this insect must be generated. Here, we report on progress made with studying *B. tabaci* egg development. *B. tabaci* has a unique reproductive system; the males develop from unfertilised eggs (haploid) and females from fertilised eggs (diploid). Using knowledge gained from the extensively characterised *Drosophila melanogaster* egg development stages, we initiated a comparative analysis to dissect processes involved in the early egg development of *B. tabaci* with a focus on the pre-blastoderm to cellularization stages. Further insights into egg developmental biology can help optimise research tools such as germline transformation, which has a potential for new genetic methods of control.

Keywords: *Bemisia tabaci*, embryonic development

PO485

ABA-INDUCED CHANGES ON TOTAL AND DIFFERENTIAL HEMOCYTE COUNTS OF *GALLERIA MELLONELLA* L. (LEPIDOPTERA:PYRALIDAE)

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Plant growth regulators are a group of plant derived compounds that regulate plant biochemistry and physiology. They are also recommended as chemosterilants against insect pests and have the potential to use in integrated pest management programs. Abscisic acid (ABA) is one of the five classes of phytohormones and proposed as a defense regulator in plants and an endogenous immune modulator in animals. ABA also known to have JH-mimicking effects as both ABA and JH are derived from the same precursor. To further clarify this we directly injected different concentrations of ABA below LD50 (10, 20, and 50 mg/ml) into the hemolymph of *Galleria mellonella* L. (Lepidoptera: Pyralidae) and observed changes on total and differential hemocyte counts as indicators of immune function. Injection of different concentrations of ABA resulted in a remarkable decrease in the number of circulating hemocytes at 4, 24, and 48 h post-injection. The minimum count of $12.11 \pm 1.0 \times 10^6$ cell/ml was observed at 48 h after ABA injection at the highest concentration of 50 mg/ml, whereas a maximum count of $21.87 \pm 1.4 \times 10^6$ cell/ml was recorded at the lowest dose of 10 mg/ml. Larvae that were injected with 10 mg/ml and 50 mg/ml ABA displayed significantly higher number of granulocytes at 4 and 24 h, but a sharp reduction at 50 mg/ml, 48 h post injection. The largest decrease in plasmatocyte ratio was observed at 50 mg/ml at 24 h post-injection compared to controls and other doses. According to our data we speculate that ABA may interfere with the cellular immunity of insects via its effect on total and differential hemocyte counts.

Keywords: *Galleria mellonella*, ABA, hemocyte counts, immunity

PO486

INFLUENCE OF AZADIRACHTIN ON PRE-ADULT DEVELOPMENT OF *GALLERIA MELLONELLA* L. (LEPIDOPTERA: PYRALIDAE)

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The effects of the botanical insecticide azadirachtin were examined on some biological parameters (cocoon formation time, last instar-to-pupal developmental time, pupal period, percentage of pupation and adult emergence) of *Galleria mellonella* L. (Lepidoptera: Pyralidae). Different concentrations of azadirachtin (1, 5, 10, 50, 100, 500, 1000, 3000, and 10000 ppm) was applied topically to the final instar larvae of *G. mellonella* to be tested for the evaluation of time taken for cocoon formation and the PD50 (the median pupation dose) value. Treatment of azadirachtin gave a PD50 of 73.088 ppm and all individuals were dead at doses >100 ppm according to cocoon formation time observations. Azadirachtin slightly increased and decreased the cocoon formation time and the pupal period of *G. mellonella* compared to control groups. However, azadirachtin treatments noticeably extended the last instar-to-pupal developmental time of *G. mellonella* at all doses, but the delay of developmental time was only significant at 10 and 100 ppm according to controls. When compared experimental groups with each other, the last instar larvae at 100 ppm pupated significantly longer time than larvae at 1, 5 and 50 ppm. Although all the individuals of control groups pupated, percentage of pupation markedly decreased at different rates depending on the dose of the chemical. Similarly, the percentage of adult emergence showed insignificant reductions at doses >1 ppm compared to controls. Therefore, azadirachtin did not significantly affect the percentage of pupation and adult emergence of *G. mellonella* but the lower rate of adult emergence of pupated individuals suggests that the chemical could be toxic for this moth. The overall evaluation of current observations showed that azadirachtin has the potency to affect the model insect *G. mellonella*.

Keywords: *Galleria mellonella*, azadirachtin, toxicity, biological parameters

PO487

PRELIMINARY BIOASSAY ON TOXICITY OF *BACILLUS THURINGIENSIS* APPLIED TO *GALLERIA MELLONELLA* L. (LEPIDOPTERA: PYRALIDAE)

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Bacillus thuringiensis is a widespread entomopathogenic bacterium that produces endospores called Cry and Cyt toxins during the growth phase. These protein toxins are selective to their target insect, innocent to plants and vertebrates, and highly biodegradable therefore *B. thuringiensis* is successfully applied as a bioinsecticide for the control of insect pest in the agriculture. When ingested by the larvae, the toxins dissolve in the midgut and activated. The primary action of activated toxins is to lyse midgut epithelial cells in the target insect by forming lytic pores on the cells. This effect may allow bacterial cells to enter insect haemocoel, resulting in development of septicaemia and death. In this study, last instars of *Galleria mellonella* L. (Lepidoptera: Pyralidae) were force fed with 0.6×10^6, 1.2×10^6, and 2.4×10^6 CFU/ml *B. thuringiensis* suspension for the evaluation of lethal concentration along with a control group. Control and experimental insects that force fed with ten µl of each concentration were held in sterile Petri dishes, and observed daily to determine the percentage of mortality. According to mortality data some chosen LCx values of B.thuringiensis with associated 95% confidence levels were determined using probit analysis in SPSS software. The highest percentage mortality was observed as 42% at 2.4×10^6 CFU. LC50 was determined as 3.867×10^6 CFU, while LC10 value for larvae was 0.110×10^6 CFU. According to probit analysis, all the used concentrations of *B. thuringiensis* showed insecticidal activity on *G. mellonella* larvae.

Keywords: *Galleria mellonella*, *Bacillus thuringiensis*, toxicity, mortality

PO488

EGG SIZE AND OFFSPRING PERFORMANCE OF THE PINE-TREE LAPPET *DENDROLIMUS PINI*

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It is assumed that larger females tend to produce more numerous and larger offspring. Recent meta-analysis found that across a great diversity of species, maternal size showed a positive correlation with both offspring size and number, but there is no theoretical consensus on why this is so. This pattern may likely be driven by variation in maternal nutrition and life conditions. For the pine-tree lappet moth (*Dendrolimus pini*), the earliest environmental experience comes via maternal nutrients supplied to the egg. Egg size is an important life-history trait because it affects hatching time, survival, duration of development, and larval size. During study, we noticed despite feeding the larvae (separate offspring of different females) of the pine-tree lappet, with the same food, there are differences in the body size and rate of growth and development of offspring. However, the high diversity in larval size and in development rate are present not only between the sexes (adults shows sexual size dimorphism) but especially within the same sex. The aim of study was to determine whether egg size can differentiate the rate of growth and development of the pine-tree lappet. We verified the following hypothesis: larger pine-tree lappet parents produce larger eggs, and larvae which emerge from larger eggs are characterised by larger body size and faster growth and development. We noticed that the mass of oviposited eggs gradually declined in the later days of the oviposition process. The effect of egg size on both growth development of the larvae and adults parameters was against our expectations. It turned out that diverse resource allocation to eggs is not a reason of differences in the body size and rate of growth and development of offspring, because from medium-sized eggs, the offspring were the largest and the fastest growing.
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Keywords: Growth and development, life-history traits, pine pests

PO489

LEVELS OF ENCAPSULATION AND MELANIZATION IN *GALLERIA MELLONELLA* (LEPIDOPTERA: PYRALIDAE) LARVAE EXPOSED TO TITANIUM DIOXIDE NANOPARTICLES

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Titanium dioxide nanoparticles (TiO2 NPs) are used in great varieties of fields such as food, cosmetic and paint industries and medical applications. However, toxic effects of TiO2 NPs have not known thoroughly. The greater wax moth, *Galleria mellonella* is a model organism for biological, physiological and toxicological studies because of the fact that can easily be cultivated in laboratories. In our study, levels of encapsulation and melanization in *G. mellonella* larvae were fed with honeycombs loaded with doses of 100, 500, 1000, 3000, and 5000 ppm TiO2 NPs from the first larval stage were investigated. To determine the effects of TiO2 NPs to the cellular responses of the larvae, the Sephadex A- 25 beads were injected in to the haemoceol of last instar larvae and then we observed the levels of encapsulation and melanization around beads. Through the application of TiO2 NPs, increased rate of strongly encapsulated beads at most of doses were determined at 4h post- injection. We found that the level of encapsulation at all treatment groups were similar. Decreased rate of weakly melanizated beads at 100, 1000 and 5000 ppm doses at 4h post injection were obtained. The rate of weakly melanizated beads increased while the rate of strongly melanizated beads decreased at all doses except 100ppm at 24h post- injection. The results show that TiO2 NPs may alter the immune responses of *G. mellonella*.

Keywords: Titanium dioxide nanoparticles, *Galleria mellonella*, Encapsulation, Melanization

PO490

APOPTOSIS AND NECROSIS IN HEMOCYTES OF *GALLERIA MELLONELLA* L. (LEPIDOPTERA: PYRALIDAE) DUE TO ALUMINIUM TOXICITY

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Aluminium is generally known as well tolerated by animals. However, biological effects of aluminium on animals have not been clear yet. The Greater Wax Moth, *Galleria mellonella* is an important pest for apiculture since larvae feed on honeycomb inside bee nests. On the other hand, *G. mellonella* is an excellent model organism for *in vivo* toxicity testing. Apoptosis and necrosis are the major processes by which cells die. The aim of this study is to determine the possible effects of aluminium on the frequency of apoptotic and necrotic hemocytes in *G. mellonella* larvae. The cultivation of *G. mellonella* was conducted in the laboratory at 27±1°C, 60±5% relative humidity and dark conditions. To investigate the effects of different doses of Aluminium chloride (Aluminium chloride hexahydrate, Merck) (10.000, 25.000, 50.000 ve 100.000 ppm), selected last instar larvae which were 0.16±0.01 g in weight were fed by insect force feeding method with a Hamilton syringe. To determine the occurrence of apoptosis and necrosis the method via acridine orange and ethidium bromide double staining of the hemolymph was applied at 48h after force feeding. We found that, the ratio of late apoptosis and necrosis increased significantly in all aluminium treated groups. The results show that aluminium has negative effects on hemocytes of *G. mellonella*.

Keywords: Aluminium toxicity, *Galleria mellonella*, Hemocyte, Apoptosis, Necrosis

PO491

EFFECTS OF ZINC OXIDE NANOPARTICLES ON LEVELS OF ENCAPSULATION AND MELANIZATION IN *GALLERIA MELLONELLA* (LEPIDOPTERA: PYRALIDAE) LARVAE

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Zinc oxide nanoparticles (ZnO NPs) are widely utilized in different industrial sectors. Previous studies showed that ZnO NPs have cytotoxic effect. The mechanisms of cytotoxicity from ZnO NPs have not been completely determined yet. Recently, studies on the effects of ZnO NPs on insects as model organisms have gained importance. This study was undertaken to investigate if ZnO NPs have any effect on the encapsulation and melanization responses of the greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae). Different doses of ZnO NPs (100, 500, 1.000, 3.000, 5.000 ppm) added to larval diet and larvae were fed with the diet from first stage until last stage. To determine the effects of ZnO NPs, the Sephadex A- 25 beads were injected in to the haemoceol of last instar larvae and then the levels of encapsulation and melanization around beads were observed. The results showed that the rate of weakly encapsulated beads increased at 4h post- ZnO NPs injection into larvae. The rate of melanization increased at 4h post- injection while decreased at 24h. However the level of melanization was obtained from the control group was higher at 24h than 4h post-injection. As a result, ZnO NPs caused changes in immune response of *G. mellonella* larvae.

Keywords: Zinc oxide nanoparticles, *Galleria mellonella*, encapsulation, melanization

PO492

EFFECTS OF AZADIRACHTIN ON DEVELOPMENT OF *GALLERIA MELLONELLA* L. (LEPIDOPTERA: PYRALIDAE)

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It is known that Azadirachtin (AZA), a plant origin substance, is used against pest organisms and lepidopteran species are quite sensitive against this substance. In this study, effects of AZA on development of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae via force feeding were investigated. The technical AZA (Sigma, St. Louis, MO) was used for experimental analysis. To investigate the effects of different doses of AZA (0.2, 0.5, 1, 1.5, 2, 3, 5 µg/larva), selected larvae which were 0.16±0.01 g in weight were fed by insect force feeding method. Larvae were starved for 3 h before treatment and then were force fed by applying 5 µl of AZA solution with a Hamilton syringe. After injection, each larva was maintained in a sterile plastic box containing 2 g diet at 27±1°C, 60±5% RH, and was observed daily. The results showed that all doses of AZA showed insecticidal activity on *G. mellonella* larvae. Larval developmental time increased while that of pupa did not change depending on increasing doses of AZA. On the other hand, adult longevity, and weights of pupae and adults decreased. Our results show that AZA effects as an insecticidal agent on *G. mellonella* larvae studied as a pest model organism.

Keywords: Azadirachtin, *Galleria mellonella*, survival time, weight

PO493

CHANGES IN THE TOTAL HEMOCYTE COUNT OF *GALLERIA MELLONELLA* L. (LEPIDOPTERA: PYRALIDAE) LARVAE CAUSED BY COLD EXPOSURE

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Hemocytes are very vital component of the insect immune system. Studies on immune system of insect pest species under altered conditions are important for pest management strategies. Insects are cold-blooded, and their physiology is greatly influenced by the temperature. Most insect species exposed to low temperature can survive but some physiological changes may occur. The greater wax moth, *Galleria mellonella* is an excellent

model organism for studying on immune system. In this study, selected last instar larvae which were 0.16±0.01g in weight were transferred from laboratory conditions (27±1°C and 60%RH) to climate chamber (10°C and 60%RH). After exposure to 10°C for 3, 5, and 10 days separately, hemolymph samples were obtained by puncturing larval abdomen with a sterile needle. The out-flowing hemolymph was transferred into Eppendorf tubes containing anticoagulant solution to prevent melanisation. Number of hemocytes were counted on the Neubauer hemocytometer under a light microscope. We found that the total hemocyte count decreased at 3 days cold-exposed group. The total hemocyte counts of larvae exposed to low temperature for 5 and 10 days were found similar to control group. These results show that low temperature may negatively affect the immune system of *G. mellonella* larvae but larvae may physiologically adapt to cold acclimations for longer durations.

Keywords: Immune system, Low temperature, *Galleria mellonella*, Hemocyte

PO494

EPIGENETIC MODIFICATIONS PLAY KEY ROLES IN JUVENILE HORMONE ACTION

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Epigenetic mechanisms including DNA methylation and post-translational modifications of histones are emerging as key determinants of hormone action. Antagonistic actions of histone acetyltransferases (HATs) and histone deacetylases (HDACs) mediate transcriptional reprogramming during the development of *Drosophila melanogaster* and other insects. Juvenile hormones (JH) play crucial roles in the regulation of growth, development, metamorphosis, reproduction and other important physiological processes. However, our current understanding of epigenetic regulation of JH action is still limited. Hence, we studied the role of CREB binding protein (CBP, contains HAT domain) and Trichostatin A (TSA, HDAC inhibitor) in JH action using RNA seq based transcriptomic analysis. Exposure of TcA cells to JH caused a rise in the expression of Kr-h1 (a well-known JH-response gene) and 32 other genes. RNAi mediated Knockdown of CBP caused a decrease in the expression of 456 genes including Kr-h1 whereas exposure to TSA resulted an increase in expression of 699 genes in TcA cells compared to the cells exposed to control dsRNA targeting a gene in *E. coli* (dsmalE). Intriguingly, expression of several genes coding for transcription factors, nuclear receptors, P450s and fatty acid synthase family members that are known to mediate JH action were responded to CBP knockdown or TSA treatment. These data suggest that acetylation and deacetylation mediated by HATs (CBP) and HDACs (TSA) play an important role in JH action. Further studies based on chip sequencing and functional proteomics will help to elucidate the exact mechanisms involved in epigenetic regulation of JH action in insects.

Keywords: HAT, HDAC, Kr-h1, TcA cells, RNA seq, RNAi.

PO495

PRODUCTION AND ACTIVITY EVALUATION OF *HELIOTHIS VIRESCENS* RECOMBINANT PROTHORACICOTROPIC HORMONE

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The prothoracicotropic hormone (PTTH) is a neuropeptide that, interacting with the TORSO receptor, stimulate prothoracic glands (PGs), triggering a cascade of events that lead to the synthesis of ecdysone, the molting hormone. It is produced in neurosecretory cells of the brain together with other hormones (insulin-like factor and prothoracicostatic hormone, the natural PTTH antagonist). PTTH is synthesized as pre-pro-hormone and subsequently processed in the brain, to obtain a glycosylated homodimer with active subunits of 109 amino acids, with intra- and extra-chain disulfide bonds. The study of ecdysteroidogenesis by *in vitro* incubation of PGs is generally performed using a crude brain extract. This method does not allow to standardize the dose and amount of PTTH to be used in incubation tests, since the brain homogenate represents a complex matrix of components with unknown relative proportions which make the results of such experiments highly variable. In this work, the recombinant active form of PTTH (rPTTH) of the tobacco budworm *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) has been produced. The main goal is to obtain a purified rPTTH suitable to perform *in vitro* tests, in a standardized and repeatable manner. Avoiding the use of the crude brain extract would allow determining the real dose of rPTTH which is required to synthesize and secrete ecdysone. We evaluated the functionality and stimulatory effect of rPTTH by *in vivo* and *in vitro* tests on *H. virescens* last instar larvae PGs.

Keywords: *Heliothis virescens*, Recombinant prothoracicotropic hormone, prothoracic glands, ecdysteroidogenesis

PO496

ENERGY BUDGETS AND METABOLIC CHANGES DURING EXTENDED DIAPAUSE IN THE SPRUCE WEB-SPINNING SAWFLY *CEPHALCIA ABIETIS*

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Cephalcia abietis is a forest defoliator on Norway spruce; larvae develop in the tree crowns and feed old needles. Mature larvae drop to the ground and burrow a chamber in the soil for overwintering. Development of the insect to maturity depends on soil temperatures. Eonymphs develop into pronymphs either in the same year or enter a prolonged diapause as eonymphs for several years. Pronymphs require a chilling period (winter) before they turn into pupae the following spring and emerge as adult sawflies. Thus, the insect spends most of its life cycle in the soil without feeding and relies on engery reserves accumulated during larval feeding. The present study examined the biochemical changes and metabolic adjustments of the developmental stages (eo- and pronymphs, pupae, adults) during the three-year life cycle of a *C. abietis* population in Austria. Mature larvae were trapped in funnels, eonymphs, pronymphs and pupae were collected from the soil and adult wasps were caught with nets. The temporal changes in insect body mass, water, protein, lipid, carbohydrate and glycogen contents were recorded and the supercooling abilities and respiration rates were measured. All developmental stages were freeze-avoiding; the overwintering/diapausing eonymphs and pronymphs exhibited a high ability to supercool and a very low oxygen consumption that were maintained throughout the years. During pupation, the supercooling ability decreased significantly while respiration rates increased. The body water content increased from eonymphs to pronymphs and was highest in pupae. During the second overwintering cycle, eonymphs lost 25% of their body mass, diapausing pronymphs in the last winter lost only 10%. Metamorphosis resulted in a further loss of body mass. A significant drop in lipids and carbohydrates (sugar, glycogen) was observed when eonymphs developed to pronymphs and pupae, indicating that the insect utilizes these energy reserves. Protein levels did not significantly decline during dormancy.

Keywords: Diapause, energy budget, supercooling, respiration, *Cephalcia abietis*

PO497

MORPHOLOGICAL ASPECTS OF THE SPERMATECA OF A PREDATORY LADYBIRD BEETLE

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The predatory ladybird beetle *Tenuisvalvae notata* (Coleoptera: Coccinellidae) (Mulsant) is a polygamous species native to South America that has been used in either classical or natural biological control of mealybugs (Hemiptera: Pseudococcidae). The morphology as well as the storage capacity of seminal fluid in the spermatheca of *T. notata* females may affect its reproductive performance. Thus, the present study evaluated the spermatheca morphology of virgin and mated *T. notata* females using light and scanning microscopy. We also looked at the histochemistry of the spermatheca material, and to detect protein compounds we used the Xylidine Ponceau staining technique, whereas for neutral polysaccharides the Periodic Acid Schiff (PAS) was used. Results show that the spermatheca of *T. notata* is rhiniform and consists only of the receptacle and spermatic duct, being morphologically similar in virgin and mated females. There was no secretion in the spermathecae of virgin females, and in once-mated females it was not possible to observe the presence of spermatozoa. In contrast, in females mated multiple times, it was possible to observe spermatozoa in the lumen of the spermatheca surrounding the secreted material. The histochemical analyses of the spermathecae of once- or thrice-mated females revealed a positive reaction to neutral carbohydrates and total protein content. Additionally, images of the spermatheca lumen revealed that there were carbohydrates and proteins associated with the secretion and spermatozoids found in the spermatheca of mated females. It is possible that polygamy in *T. notata* is related to the maintenance of viable spermatozoa in the spermatheca, in which case the female would prefer to copulate more times during its adult life than to store spermatozoa for a longer period of time.

Keywords: Coccinellids, seminal receptacle, ultrastructure, histochemistry

PO498

FUNCTION ANALYSES OF TEN SMALL HEAT SHOCK PROTEIN GENES AND TRANSCRIPTOME ANALYSIS OF HSP18.3 USING RNA-SEQUENCING IN *TRIBOLIUM CASTANUEM*

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Small heat shock proteins (sHSPs) acted as molecular chaperones are widely distributed in all organisms, which protect organisms and cells from various stresses. To better understand the evolution and functions of shsps in insects, ten shsps have been identified from the *Tribolium castaneum* genome, in which hsp21.8a and hsp21.8b were orthologous genes among insects, and the others were *T. castaneum* species-specific shsps. All of the 10 shsps were expressed in different developmental stages, and were up-regulated in response to heat stress but not to cold stress. These genes were classified into three groups by sensitive degree to heat stress: such as strong response, medium response and weak response types. To explore their specific functions in development and fecundity, RNA interference (RNAi) experiments were carried out and revealed that all the 10 shsps could affect the beetle's development and reproduction to varying degrees. Among of which, hsp18.3 not only strongly responded to heat stress, but caused the highest mortality and the worst fecundity for beetles once it was knockdown. Moreover, ds-hsp18.3 shortened adult survival days under starvation. Thus hsp18.3 was demonstrated having critical functions on thermal stress, starvation stress, eclosion and reproduction, and then transcriptome analysis of hsp18.3 using RNA-sequencing were performed. Totally, 569 differentially expressed genes were identified between ds-hsp18.3 and control groups, which suggested that hsp18.3 may through suppressing the antioxidant activity process and amplifying serine protease signal pathway to regulate stress responses and innate immunity as well as development and reproduction of the beetles. This research will help us understand more function of insect shsps and lay the foundation for elucidating the signal transduction mechanism of hsp18.3.

Keywords: Shsps, hsp18.3, Heat stress, Development, Reproduction, *Tribolium castaneum*

Sensory System

PO499

ANTENNAL PROTEIN PROFILE IN HONEYBEES: CASTE AND TASK MATTER MORE THAN AGE

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Olfaction and chemical communication are fundamental for coordination in insect societies, especially in species forming large colonies. Reproductive and task partitioning suggest that colony members belonging to different castes or subcastes or performing different tasks during their life (polyethism) may produce different semiochemicals and be differently sensitive to the variety of pheromones and/or environmental odours. The main olfactory organs are the antennal chemosensilla, where the early olfactory processes take place. At this stage, members of two different families of soluble olfactory proteins (Odorant Binding Proteins -OBPs-, Chemosensory Proteins -CSPs-) show a remarkable affinity for different odorants and act as carriers while a further family, the Niemann-Pick type C2 proteins (NPC2) may have a similar function. Sensillar lymph also contains Odorant degrading enzymes (ODEs) which are involved in inactivation through degradation of the chemical signals, once the message is conveyed. Despite their importance in chemical communication, poor is known about how these proteins and, more generally antennal proteins, differ in honeybees of different caste, task and age. Here first we investigate, using a shotgun proteomic approach, the antennal profile of honeybee of different castes (queens and workers) and of workers performing different tasks (nursers, guards and foragers) by controlling for the potential confounding effect of age. Regarding olfactory proteins, major differences were observed between queens and workers, some of which were found to be more abundant in queens (OBP3, OBP18, NPC2-1) and others to be more abundant in workers (OBP15, OBP21, CSP1, CSP3); while between workers performing different tasks, OBP14 was more abundant in nurses with respect to guards and foragers, and two ODEs were more abundant in guards with respect to workers of 2nd week. More generally we have found that the antennal proteomes are mainly characterized by castes and tasks, while age has a limited effect on protein profile.

Keywords: Nurses, guards, foragers, shotgun-proteomics, Odorant-binding proteins, Chemosensory proteins

PO500

ODORANT BINDING PROTEINS IN THE VETCH APHID *MEGOURA VICIAE* (HEMIPTERA: APHIDIDAE) DISPLAY DISTINCT EXPRESSION PATTERNS

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Insect odorant binding proteins (OBPs) are required for chemoreception and play a key role in transporting hydrophobic semiochemicals across the lymph in chemosensilla to the olfactory receptors (ORs). To investigate the putative roles of chemosensilla involved in the selection of the host plant, scanning electron microscopy (SEM) was performed on antennae, legs, mouthparts and cauda of the adult vetch aphid *Megoura viciae* (Buckton), an economically important broad bean pest. In order to identify chemoreception candidate transcripts, RNA-seq was performed on *Megoura viciae* antennae. The *de novo* transcriptome assembly resulted in 43,251 predicted transcripts. The assembled data were used in the annotation of candidate OBP genes, on the basis of a hand-curated database of insect OBP proteins, leading to the identification of ten candidate OBP genes. Additionally, the differential expression patterns of the identified OBPs, in different body parts and at different developmental stages, were measured by real-time qRT-PCR. MvicOBP1, MvicOBP3, MvicOBP6, MvicOBP7 and MvicOBP8, whose antibodies were already available, were selected for further experiments of whole-mount immunolocalization on different tissues of the aphid (antennae, mouthparts, cornicles and the cauda). Moreover, the behavioral response of *M. viciae* to the components of the alarm pheromone (E)-β-farnesene, (±)-α-pinene, β-pinene, (-)-α-pinene, (+)-limonene and a mixture composed by (E)-β-farnesene, (-)-α-pinene and β-pinene was investigated using a glass Y-tube olfactometer. The obtained outcomes offer a chance to discuss OBPs: the connection between tissue-specific OBPs, their differential expression and their putative role in *Megoura viciae* chemoreception. The understanding of the molecular mechanisms underlying olfactory perception in this aphid may lead to the development of new biological control strategies for this pest.

Keywords: Chemoreception, odorant binding proteins, RT-qPCR, immunolocalization

Insect Metabolism and Nutrition

PO501

ZEBULARINE INDUCED ALTERATIONS IN ANTIOXIDANT ENZYME ACTIVITY AND LIPID PEROXIDATION IN MODEL HOST *GALLERIA MELLONELLA*

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Methylation is a process that adds a methyl group to a cytosine residue of DNA to convert it to 5-methylcytosine. The process of methylation is mediated by DNA methyl transferases. Zebularine is a cytidine analog that forms a covalent complex with DNA methyltransferases, which reactivates hypermethylated genes in yeast and solid tumor cells, has antitumor effects in mouse xenografts, and shows a preferential effect on cancer cells. We aimed to investigate the effects of various doses (0.25-32 mg/ml) of Zebularine on antioxidant enzymes activity and lipid peroxidation in *Galleria mellonella* L. (Lepidoptera: Pyralidae) last instars, which is an ubiquitous pest of honey bee colonies. Laboratory colonies of the greater wax moth, *G. mellonella* were established from adults reared at 25±5°C, 60±5% RH, and with a photoperiod of 12: 12 (L:D) h. SOD activity was determined using commercial available assay kits. Absorbance was read in a microtiter plate and determined at 450 nm using xanthine and xanthine oxidase systems. GST activity was determined with 1-chloro-2,4-dinitrobenzene and reduced glutathione as substrates at 340 nm for 5 min. CAT activity was estimated by measuring the decrease in absorbance at 240 nm due to hydrogen peroxide decomposition. Malondialdehyde (MDA) is an indicator of lipid peroxidation and was determined using commercial available assay kits. MDA-Thiobarbituric acid (TBA) adduct was formed by the reaction of MDA and TBA under high temperature, and acidic conditions was measured at 540 nm. Injection of Zebularine in last stage *G. mellonellae* larvae resulted in a considerable increase in MDA level at all doses but not in antioxidant enzyme activities. Two possible reasons were predicted for these results. First, this effect could be a result of the inhibitory effect of Zebularine on DNA methylation in *G. mellonella*, which leads to reducing gene

transcription in insects. Second, it is likely that high oxidative stress may cause a decreasing activity of enzymes. We are planning to monitor gene expression fold of antioxidant enzymes and find out the real impact of Zebularine on *G. mellonella*.

Acknowledgement: This research was supported by grant (2015-215Z079) from The Scientific and Technological Research Council of Turkey.

Keywords: Antioxidant enzymes, DNA methylation, *Galleria mellonella*, Zebularine

PO502

A SOLUBLE PYROPHOSPHATASE REGULATING POLYPHOSPHATE METABOLISM IN THE HARD TICK *RHIPICEPHALUS MICROPLUS*

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Polyphosphates have been found in all cell types examined to date and play diverse roles depending on the cell type. In eukaryotic organisms, polyphosphates have been investigated mainly in mammalian cells, and there are only few studies on arthropods. Pyrophosphatases were shown to regulate polyphosphate metabolism, however these studies were restricted to trypanosomatids. The present paper focuses on the tick *Rhipicephalus microplus*, a hematophagous ectoparasite that is highly harmful to cattle. We produced a recombinant *R. microplus* pyrophosphatase (rRmPPase) with the aim of investigating its kinetic parameters using polyphosphates as a substrate. Molecular docking assays of RmPPase with polyphosphates were also carried out. The kinetic and Hill coefficient parameters indicated that rRmPPase presents greater affinity, higher catalytic efficiency, and increased cooperativity for polyP15 than for polyP3. Through molecular docking it was observed that polyP3 binds close to Mg2+ atoms in the catalytic region of the protein, participating in their coordination network, whereas polyP15 interactions involve negatively charged phosphate groups and basic amino acid residues, such as Lys56, Arg58, and Lys193; polyP15 has a more favorable theoretical binding affinity than polyP3, thus supporting the kinetic data. This study shows, for the first time in arthropods, a pyrophosphatase with polyphosphatase activity, suggesting its participation in polyphosphate metabolism.

Keywords: Metabolism, polyphosphate

PO503

SMALL SCALE GERM-FREE REARING OF THE SILKWORM ON ARTIFICIAL DIET

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The sericulture laboratory of CREA developed an artificial diet (patented in 2004) to rear silkworm larvae; this achievement has the aim of overcoming the constraints due to the limited leaf sprouting season of the mulberry (the unique silkworm natural food) in temperate countries and the possible pollution problems due to the leaf accidental contamination by pesticides. This diet is added with antimicrobials to limit infections and consequent epidemic disease spreading that might be caused by possible silkworm pathogens. However, the onset of bacterial resistance towards the antimicrobial additives was observed quite immediately. In particular, a Gram positive bacterium, *Enterococcus mundtii* has often been responsible of spreading of “flacherie”, an infectious disease, among the silkworm larvae reared on artificial diet. The new idea was to substitute the use of antimicrobials and to prevent contaminations by employing a small scale germ-free rearing system. This technique, already used in Japan, on a large scale in cleanrooms for silkworm rearing (Matsubara method), was transferred into a lab-scale, for rearing in closed plastic boxes containing the diet, which is autoclaved in the boxes themselves and sterilized before introducing silkworm larvae. The method is particularly apt for the laboratory standardized conditions, and for microbiological studies, because it permits the controlled infection of larvae; however, it might be also advantageously used for commercial purposes, especially for marketing of living preys for pet animals. This system was patented in 2015 (“Metodo per allevare larve del baco da seta e suoi usi derivati”).

Keywords: Silkworm, *Bombyx mori*, artificial diet, germ-free rearing

PO504

SUGAR FEEDING REDUCES HOST-SEEKING BEHAVIOUR IN THE MOSQUITO *AEDES ALBOPICTUS*

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Taking a blood meal is a key step in the lifecycle of female anautogenous mosquitoes, since it is essential for egg development. Importantly, viruses and/or other pathogens, such as Plasmodium, can be transferred from the mosquito to humans through the saliva of the insect, which is injected into the host to facilitate engorgement with blood. Therefore, it is crucial to understand the underlying molecular mechanisms controlling mosquito host-seeking behaviour, as this could lead to the development of novel vector control strategies that reduce the mosquito appetite for blood. In this context, our research demonstrates that the nutritional status of an adult female mosquito has a severe impact on host-seeking behaviour. Specifically, we show that sugar feeding strongly inhibits host-seeking behaviour in *Aedes albopictus* females, an effect that is reminiscent of the well-known feeding inhibition induced by a blood meal until oviposition. Sugar is a key food source of adult mosquitoes, including females, as it is needed to replenish energy reserves necessary for survival, flight and reproduction. The observed inhibition of host-seeking behaviour is dependent on both the type of sugar the mosquitoes have access to and its concentration. Using high-throughput RNA-sequencing, metabolite quantification and functional studies via RNA-interference, we uncover the molecular pathways which respond to sugar feeding and play a role in regulating the host-seeking behaviour of female mosquitoes. We expect that these pathways are also controlled by blood feeding, possibly indicating common mechanisms that can be targeted to reduce mosquito appetite and aggressiveness.

Keywords: *Aedes albopictus*, host seeking behaviour, sugar feeding, functional studies

PO505

CHARACTERIZATION OF EYE COLOUR MUTANT STRAINS (YELLOW, WHITE) OF HOUSE CRICKET (*ACHETA DOMESTICUS*)

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For many years strains of eye colour mutants of *Drosophila melanogaster* were commonly used as a basic model in physiology and molecular biology of insects. Available data reports that genes responsible for pigments synthesis influence other phenotypic traits. They affect various aspects of insect physiology and behaviour like phototaxis, locomotion, aggression, metabolism or memory. In 2015 in our department (Faculty of Biology and Environment Protection, University of Silesia) two strains of eye-coloured mutants were isolated - yellow and white strain of house cricket (*Acheta domesticus*). There is almost no data describing similar mutation in crickets, and there is lack of data about the behavioural effect of this type of mutation in hemimetabolic insects. In our research, we demonstrated deficits of two main screening pigments in eyes. Yellow-eyed crickets have no ommochromes, while the white eye crickets lack ommochromes and pteridines. This may suggest that tryptophan metabolism pathway is dysfunctional, what affects the functioning of the insect body. This pathway is responsible for the synthesis of ommochromes, but also serotonin, melatonin and other bioactive compounds. Results of genetic crosses confirmed that for both mutations, independently inherited genes were responsible. Observed phenotypes are not coupled with sex. Tests on mutants show many physiological and behavioural dysfunctions, probably caused by mutation. We identified changes in eye structure, development, lifespan, fertility, reaction to stress and anaesthesia, aggression and locomotor activity. There is a potential to use those strains as a models in research on hemimetabolous insect physiology and biochemistry. Results of experiments on mutant strains will be presented.

Keywords: House cricket, eye colour mutant, ommochromes, pteridines, tryptophan

PO506

MOLECULAR MECHANISMS UNDERLYING ACIDIFICATION AND ALKALIZATION ALONG THE LARVAL MIDGUT OF *MUSCA DOMESTICA*

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M. domestica larval anterior (A), medium (M) and posterior (P) midgut pH are, respectively: 6.1, 3.1 and 6.8. Acidification seems to depend on H+-ATPase and carbonic anhydrase and alkalinization, of ammonia (Terra and Regel CBP 112A (1995): 559-564). To disclose the molecular mechanisms involved in luminal midgut buffering, we used RNA-seq analyses from samples of sections of the midgut (A1, A2, M1, M2, P1, P2, P3) to evaluate the expression levels of chosen genes. Transporters were checked as being apical or basal by proteomics of microvillar preparations. Two HCO3-/Cl- exchangers were more expressed at M1; two H+-V-ATPases, 2 carbonic anhydrases, and one Na+/H+ antiporter were more expressed at M2; a chloride channel (Glu activated) at M1, M2, and P1; one NH3/NH4+ exchanger, two major proton-coupled amino acid transporters and a proton-peptide transporters at P1-3. The data suggest the following model: acidification occurs at M2 by the action of H+-V-ATPase with protons produced by a carbonic anhydrase, followed by chloride ions through a channel. The pH at this region is attenuated by a Na+/H+ exchanger. Alkalization at P1 results from the action of NH3/NH4+ exchanger and proton-coupled amino acid and peptide transporters, all removing protons from the lumen. The model is supported by previous finding that acetazolamide (inhibitor of carbonic anhydrase) increases the pH in middle midgut and also by the finding of large amounts of chloride in middle midgut and ammonia in posterior midgut. The model is also supported by the decrease of M1 pH (from 2.49 to 1.86) in the presence of the Na+/H+ exchanger inhibitor amiloride; the decrease in P1 pH (5.53 to 3.12) with dimethylammonia (inhibitor of NH3/NH4+ exchanger) and increase of P1 pH (5.53 to 7.24), when hydrolyzed protein was added to the starch diet.
Supported by FAPESP, CNPq, and INCT-Entomologia Molecular

Keywords: *Musca domestica*, Membrane transporters, Midgut Acidification, Midgut Alkalinization

PO507

TOXICOLOGICAL EFFECTS OF DIMETHOATE ON SOME BIOCHEMICAL PARAMETERS OF *GALLERIA MELLONELLA* L. LARVAE

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Toxicological effects of different sublethal concentration of dimethoate (10, 25, 50, 75 and 100 µg/ml) were investigated on the amount of malondialdehyde (MDA) and antioxidant enzyme activity (catalase and superoxide dismutase (SOD)) of the larvae of greater wax moth *Galleria mellonella* L. The dilution of dimethoate (10 µl) injected into the hemocoel via leg of larvae by a Hamilton syringe. The MDA content, SOD and Catalase activity were analyzed by spectrophotometric method. We showed that sublethal concentration of dimethoate injection resulted in gradually increased the MDA contend at 24, 48, 72 and 96 hours. MDA contend increased in a dose-dependent. Although SOD and catalase enzyme activity were increased at low dimethote (10, 25, 50 µg/ml), the activity of SOD and catalase tended to decrease at high level of dimethate (75 and 100 µg/ml) compared with the control. MDA content can be used as a reliable biomarker of dimethoate contamination in invertebrate model animal *G. mellonella*.

Keywords: Dimethoate, MDA, Enzyme activity, *Galleria mellonella*

PO508

EFFECTS OF THERMAL REGIMES ON HEMOLYMPH K+ AND NA+ CONCENTRATIONS AND CARBOHYDRATE CONTENTS OF LAST INSTAR LARVAE OF THE KHAPRA BEETLE, *TROGODERMA GRANARIUM* EVERTS

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The Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) is an important insect pest of stored products. In this research, changes in concentrations of hemolymph K+ and Na+ were investigated at different thermal regimes, *i.e.* cold acclimation (CA), Fluctuated thermal treatment (FTT) and rapid cold acclimation (RCA). Our results showed that in all the regimes as well as the control, concentration of Na+ decreased whereas, concentration of K+ increased with increase in exposure time of the larvae at -10 ºC. Glycogen content in control larvae with 34.4±2.3 mg/g body weight was at the highest level and reached to the lowest level of 23.0±1.6 in cold acclimated larvae. No significant differences were observed between glycogen contents in FTT, RCA and control. Change in trehalose content was reversely proportional to change in glycogen content. The highest and lowest trehalose content was observed in CA and control, respectively. These finding reveals profound impact of cold acclimation on carbohydrate contents of the larvae. Cold acclimation play an important role in conversion of glycogen to low molecular weight carbohydrates which act as cryoprotectant to enhance cold hardiness of the larvae.

Keywords: Hemolymph ions; glycogen content; trehalose content; the Khapra beetle

PO509

EFFECT OF THERMAL REGIMES ON SURVIVAL AND CARBOHYDRATE CONTENTS OF 4TH INSTAR LARVAE OF THE KHAPRA BEETLE, *TROGODERMA GRANARIUM* EVERTS

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The Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) is an important insect pest of stored products. In this study, cold hardiness and LT80 (lower lethal temperature for 80% mortality) were investigated at different thermal regimes, *i.e.* cold acclimation (CA), fluctuated thermal treatment (FTT) and rapid cold hardiness (RCH). Our results indicated profound effect of cold acclimation on LT80 value of the larvae. In cold acclimated larvae temperature required for 80% mortality decreased about 10 ºC. In addition, temperature necessary for start of mortality in CA regime was 10 ºC lower than that of control. In all the regimes survival rate of the larvae decreased with decrease in temperature and increase in exposure time. Cold acclimated larvae showed the highest cold hardiness in the lowest temperatures of -15 and -20 ºC. The greatest myo-inositol and glucose contents were detected in larvae cold acclimation treatment (10.7±0.4 μg/dry weight) and control (0.49±0.03 μg/dry weight), respectively. By contrast, control and cold acclimation treatment larvae had the lowest myo-inositol (6.0±0.6 μg/dry weight) and glucose (0.14±0.02 μg/dry weight) contents, respectively. Also, the different treatments did not significantly affect sorbitol content of *T. granarium* larvae when compared with control larvae. Our results indicate significant enhancement of larval survival and cold tolerance under CA regime.

Keywords: Cold hardiness; cold acclimation; fluctuated thermal treatment; rapid cold hardiness

PO510

METABOLISM OF ANTS (*FORMICA PRATENSIS*) IN A VARIABLE ENVIRONMENT, DURING ACTIVITY AND REST

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Ants are poikilothermic insects, incapable of regulating the body temperature by changing their metabolic processes. Their energy requirements depend strongly on environmental conditions. In judging the impact of global temperature increase it is essential to know their metabolism both in resting and active state. The black-backed meadow ant (*Formica pratensis*) is a xerothermophilic species which prefers dry habitats like open meadows with high solar radiation. We investigated the respiration (resting and active metabolism) of the ants in the entire temperature range the ants are usually exposed to in their natural habitat. At each ambient temperature (Ta=5°C to 45°C, 5°C steps), seven individuals were placed in single multiplex-controlled respirometer chambers where their CO_2 production was recorded in stop and go mode. The active and resting behavior were determined by observing and categorizing digital video recordings. The active individuals demonstrated a considerably higher (about 58-97%) metabolism than the resting ones. In the investigated temperature range mean CO_2 production rate of resting ants increased according to an exponential function (0.066 nl s-1 at 5°C, 0.907 nl s-1 at 20°C, 5.272 nl s-1 at 40°C), whereas active ants showed a steeper, more sigmoidal increase (0.5694 nl s-1 at 5°C, 1.228 nl s-1 at 20°C, 9.328 nl s-1 at 40°C). No resting individuals could be observed above 40°C. Our active black-backed meadow ants showed a similar CO_2 production than reported for red wood ants (*Formica polyctena*) which are related to our experimental animals but differ in nest site preference. Our results demonstrate the importance of considering locomotor activity in such measurements. The increasing ambient temperature caused by climate change poses a challenge to these insects, increasing the metabolism of both resting and active ants. This emphasizes the need for additional research to clarify the impact of critically increased temperatures on their energetics.

Keywords: Ants, metabolism, temperature, *Formica pratensis*, respiration

PO511

EFFECT OF THERMAL REGIMES ON ENZYME ACTIVITY OF 4TH INSTAR LARVAE OF THE KHAPRA BEETLE, *TROGODERMA GRANARIUM* EVERTS

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The Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) is an important insect pest of stored products. In this study, activities of four enzymes (AMP-depended protein Kinase, protein phosphatase I, protein phosphatase IIA and protein phosphatase IIC) were investigated at different thermal regimes, *i.e.* cold acclimation (CA), fluctuated thermal treatment (FTT) and rapid cold hardiness (RCH). Our results showed that the highest and lowest activities of AMP-depended protein kinase and protein phosphatase IIC were observed at CA and FTT treatments, respectively. No significant differences were observed in the activities of these two enzymes between control and CA treatments. The highest and lowest activities of protein phosphatase I was observed at control and CA treatments, respectively. In case of protein phosphatase IIA the highest level of activity was recorded for control and FTT treatments whereas, the lowest level of activity was shown in CA and RCH. These findings revealed that the shape of the thermal reaction in AMP-depended protein Kinase and protein phosphatase IIC follow a same norm that is different from those of protein phosphatase I, protein phosphatase IIA. Protein phosphatase IIA and IIC showed completely different thermal reaction norms. In did, thermal fluctuation cause the highest changes in the enzymes activities whereas, the enzymes activities showed the lowest changes in RCH.

Keywords: AMP-depended protein kinase; protein phosphatases; the Khapra beetle

PO512

HETEROGENEITY IN LARVAL DIETARY ENVIRONMENT AND ITS EFFECTS ON LARVAL FORAGING BEHAVIOUR AND FITNESS-RELATED TRAITS IN THE POLYPHAGOUS FRUIT FLY *BACTROCERA TRYONI*

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Larval foraging behaviouris a key factor underpinning individual growth and fitness. Heterogeneity in the nutritional environment at the developmental stage may result in larvae foraging in lower-than-optimal diets, with potential downstream fitness effects in the present and future generations. To date, however, the consequences of variation in larval foraging behaviour arising in heterogeneous nutritional environments to larval development and adult reproduction, as well as next generation traits, remain poorly understood. To address this gap, we allowed larval groups of the polyphagous fruit fly *Bactrocera tryoni* (aka "Queensland fruit fly") to forage freely in multi-choice foraging arenas with heterogeneous patch options, each patch consisting of a different diet with macronutrient dilutions ranging from 100% to 20%. Our results showed that larval foraging patterns are highly consistent and independent of the number of conspecifics in the foraging group. This consistency allowed us to rank larval dietary choices into preferred (100%), intermediate (80% and 60%), and unwanted diets (<60%). We then selected the preferred (100%) and intermediate (80% and 60%) diets for fitness assays, where we allowed egg-to-adult development in the diets and measured developmental traits (*i.e.* egg hatching and pupation), adult performance and reproduction traits (*i.e.* adult emergence, body mass, flight ability, fecundity), and next-generation pupal weight. The intermediate diet with 60% macronutrient concentration delayed egg hatching and pupation rate. However, larvae fed in both preferred (100%) and intermediate (80% – 60%) diets had equivalent egg hatching success (total eggs hatched), total number of pupae and pupae weight, adult emergence, body mass, flight ability and cold tolerance, fecundity, as well as offspring pupae weight. Together, our findings demonstrate that larval dietary choices only include diets that can support adult fitness. The behavioural and physiological processes that allow the nutritional flexibility of the larvae, where individuals attain fitness even when developing in reasonably moderated macronutrient dilutions, likely underpins the success of *B. tryoni* and other polyphagous species in sustaining growth and reproduction across a variety of ecological conditions.

Keywords: Larval nutrition, reproduction, trans-generation, development

PO513

WHAT DOES THE QUEEN EAT? - DETERMINING THE NUTRITIONAL INTAKE TARGETS OF *BOMBUS TERRESTRIS* AND *BOMBUS PASCUORUM* QUEENS

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Bumblebee population declines have been linked to a reduction in nutritional resources due to anthropogenic land-use intensification. However, our knowledge of the nutritional requirements of bumblebees remains limited. While initial studies have ascertained the nutritional requirements of bumblebee workers, no data exist for bumblebee queens, who represent the solitary stage of the bumblebee life-cycle, and are central to the persistence of populations. Post-hibernation, queen bumblebees must forage for themselves before founding a colony. During this key stage of the lifecycle queens must consume the correct quantity and quality of nutrients to achieve optimum fitness and subsequently produce a successful colony. Pollen is the primary source of lipid and protein for bumblebees, but its nutritional quality varies significantly across plant species. Here we tested the capability of queens from two abundant, but morphologically and phenologically distinct species of bumblebees - *Bombus terrestris*, a short-tongued, early-emerging species, and *Bombus pascuorum*, a long-tongued, late-emerging species - to regulate their protein and lipid intake. Wild-caught queens were restricted to a range of artificial diets that varied in protein:lipid ratios. By measuring the quantities of each macro-nutrient consumed, we determined the nutritional intake targets of queens of both species using the Geometric Framework Model. We then compared this to the resources available in the wild for these queens, to determine potential mismatches between optimal and available diets. We interpret our results in the broader context of bumblebee declines, and suggest nutritional interventions that could enhance queen fitness in the modern landscape.

Keywords: Bombus, queen, nutrition, diet, protein, lipid

PO514

DETERMINATION OF CPG DINUCLEOTIDS AND ITS METHYLATION PATTERN ON DIFFERENT STAGES OF *GALLERIA MELLONELLA* BY USING METHYLATION-SENSITIVE RESTRICTION ENDONUCLEASES

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The greater wax moths or honey comb moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) feeds on honeycomb inside bee nests and causes damage in apiculture. Laboratory colonies of *G. mellonella* were established from adults reared at 30°C, 60±5% RH, 24 h dark. We investigated the presence of CpG dinuclotides and their methylation status in eggs, larvae, pupae, and adults of *G. mellonella* by using HpaII, MspI, and ScrFI methylation-sensitive restriction endonucleases. These endonucleases are classic tools of DNA methylation analysis. 1µg DNA from all stages of *G. mellonella* was digested with all endonucleases and mix of MspI-ScrFI. The most commonly used restriction enzymes are the isoschizomers HpaII and MspI, which recognize the same DNA sequence 5'-CCGG-3' and cleave the DNA between the 2 cytosine residues. HpaII is blocked when either cytosine is full methylated, but cuts the outher cytosine hemimethylated. MspI is blocked only by full or hemimethylation of the outher cytosine. ScrFI recognizes the sequence 5'CC↓NGG3', where N is any one of the four bases. ScrFI is inacitivate, when the internal C within these sites is methylated. The gel analyses of the restriction pattern of HpaII, MspI, ScrFI and MspI+ScrFI on *G. mellonella* genomic DNA showed that all enzymes produced a wide range of smear. The digestion of DNA samples of egg, pupa, and adult resulted in a smear at higher molecular weight with respect to MspI+ScrFI and these differences were statistically important. On the other hand, the smear of larvae did not exhibit a statistically important difference between HpaII, MspI, ScrFI, and MspI+ScrFI products. These results confirmed the presence of CpG dinucleotides and methylation at the internal and outher cytosines in different developmental stages of *G. mellonella*.
Acknowledgement: This research was supported by grant (2015-215Z079) from the Scientific and Technological Research Council of Turkey.

Keywords: DNA methylation, Endonucleases, *Galleria mellonella*

PO515

BACTERIOCYTE REPROGRAMMING TO MAINTAIN ORGANISMAL HOMEOSTASIS UNDER NUTRITIONAL STRESS IN APHID SYMBIOSIS

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Nutritional symbiosis plays a central role in the ability of insects to thrive on unbalanced diets and in ensuring their evolutionary success. A genomic model for nutritional symbiosis comprises the pea aphid, *Acyrthosiphon pisum*, and the gamma-3-proteobacterium, *Buchnera aphidicola*, with genomes encoding highly integrated metabolic pathways. Its main food source, phloem sap, is nutritionally unbalanced and highly variable in composition thus raising the important question how organismal homeostasis is regulated under nutritional stress. To address this question, we investigated the organismal impact of deprivation of tyrosine and phenylalanine, two amino acids that are essential for aphid development and growth and for which host and endosymbiont are fully interdependent. Surprisingly, we found that the pea aphid does not show major phenotypic differences when deprived of tyrosine and phenylalanine. Transcriptome analyses of gut and bacteriocytes throughout the aphid life cycle highlighted the role of bacteriocytes as major regulators of this homeostasis. Rather than regulating the tyrosine and phenylalanine biosynthesis pathway itself, aphids displayed an alternative strategy. This involves a defined temporal sequence of molecular events that include an extensive transcriptional reprogramming of the bacteriocyte with a rapid down-regulation of genes encoding sugar transporters and genes required for sugar metabolism. Consistently, we found continued overexpression of the *A. pisum* homolog of RRAD, a small GTPase implicated in repressing aerobic glycolysis. In addition, we found increased transcription of genes involved in proliferation, cell size control and signaling. We propose that bacteriocytes are essential sensors of nutritional stress in aphid symbiosis, and that their transcriptional and cellular responses fine-tune host physiology to amino acid deprivation.

Keywords: Bacteriocyte, *Acyrthosiphon pisum*, homeostasis, nutritional stress, metabolism, transcriptional reprogramming

PO516

THE EFFECT OF STARVATION ON *LYMANTRIA DISPAR* PHYSIOLOGY AND ON ACTIVATION OF NUCLEOPOLYHEDROVIRUS FROM COVERT TO OVERT INFECTION

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Starvation is the strong physiological stress which usually affect insect at high population density, for example in natural outbreaks. *Lymantria dispar* L. is one of the most economically important forest defoliator species which induce cycling outbreaks in temperate forests of northern hemisphere. Here we studied the dynamics of physiological response of *L. dispar* larvae under the effect of starvation. Following parameters were measured: the activity of phenoloxidase (PO) in plasma of haemolymph, total haemocytes count (THC), the activity of necrosis/apoptosis in haemocytes, the concentration of dopamine in plasma of haemolymph. We also study the level of activation of covert baculovirus infection (estimated as the prevalence of infection by qPCR method) to overt infection. We do not register the assistance between the activity of PO (it was dramatically increase by the effect of starvation) and activation of covert infection. We also found the increase of haemocytes number dead from necrosis beginning from the first day of treatment when larvae were starved. Starvation leads to the activation of acute infection in most larvae individuals harboring covert virus. Also we found that in starved larvae the frequency of virus carrying was recorded in 1.5 times frequently than in control larvae even they were used from the same stock of eggmasses. Thus it is possible to use such type of provocation as insects' starvation for more accurate estimating of nucleopolyhedrovirus prevalence of covert virus infection in host population.
This work was supported by Russian foundation for basic research (grant # 15-04-08197) and Russian scientific foundation (grant # 17-46-07002)

Keywords: Gypsy moth, covert infection, baculovirus, starvation

PO517

INFLUENCE OF THE HIGH CONCENTRATION OF MONOSACCHARIDES ON DEVELOPMENT AND PHYSIOLOGY OF HOUSE CRICKET (*ACHETA DOMESTICUS*). HOUSE CRICKET AS A MODEL IN DIABETES RESEARCH

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The fruit fly, *Drosophila melanogaster* is a model organism in the studies of the origins of many human diseases, including diabetes. In the area of these studies, white-eye gene mutants are used, which allows testing an association of tryptophan with type 2 diabetes. Insulin resistance is observed in larvae treated with high glucose concentration and thereafter type 2 diabetes occur in imago stadium. White mutants are less susceptible to high sugar concentrations, which indicates the role of the white gene and tryptophan in the regulation of monosaccharide metabolism. Nevertheless, the fruit fly is an insect with holometabolous development, and the body size can often be a limitation in conducting many laboratory measurements. Usage of the new line of white-eyed house cricket (*Acheta domesticus*) mutants creates new possibilities, because of its hemimetabolous development which is much more similar to mammalian. This allows to create a better simulation of the processes of diabetes development in a growing insect. Results of the studies with the use of various concentrations of glucose and fructose in food confirmed that their high doses cause changes in the development and physiology of crickets, similar as in case of the fruit fly. Additionally, the development time of insects fed with the high glucose concentration was significantly longer in comparison to the individuals fed with the high concentration of fructose. Moreover, the white-eyed crickets react differently to high sugars concentrations compared to the wild-type. The results also indicate their lower sensitivity to the negative effects of high monosaccharides content in food, what is similar to observations made during research with white-eyed fruit flies. This shows the potential application of the white cricket line as a model for the study of type 2 diabetes.

Keywords: Diabetes, T2D, house cricket, white-eye mutants, tryptophan

PO518

EFFECTS OF THE MAIN SECONDARY METABOLITES OF PHYSALIS PLANTS ON A SPECIALIST AND A GENERALIST SPECIES OF LEPIDOPTERA

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Specialisation on Physalis plants requires the ability to tolerate withanolides, the main secondary metabolites of these plants that are known to have potent feeding deterrent, cytotoxic and immune inhibiting properties. Our research focuses on the specialised adaptation of the noctuid moth Heliothis subflexa and the closely related generalist species *Heliothis virescens*. We recently demonstrated that *H. subflexa* larvae not only exploit their host plant *Physalis peruviana* as a food source, but also benefit from the striking immune modulating activity of the plant-based withanolides. These findings raise questions on the mechanisms by which *H. subflexa* overcame the inhibitory effects of these compounds. So far, nothing is known about the fate of withanolides in the caterpillar body. Withanolides are a group of C28 steroids built on an ergostane skeleton. The high reactivity of the unsaturated carbonyl system of these anti-herbivore compounds suggests an activation after ingestion in the insect gut by *e.g.* P450 enzymes, which are known to be involved in metabolite detoxification in other lepidopteran species. Preventing this activation in *H. subflexa* larvae might explain their apparent tolerance to withanolides. We here present the results of feeding assays in which both heliothine moth species were fed with defined amounts of purified withanolides that we performed to elucidate their potential metabolism or metabolic circumvention. Subsequent analysis of the faeces, gut and the rest body via LC-MS allows us to detect differences in withanolide metabolism between both species. Further experiments will be planned to identify what causes the expected differences in withanolide conversion. Our research contributes to a better understanding of the mechanisms of specialised host-plant co-adaptation in Lepidoptera.

Keywords: Plant-insect interactions, Lepidoptera, Withanolides, Physalis, plant toxins

PO519

SPERM METABOLISM IN INSECTS - WHAT DO WE KNOW?

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Sperm are morphologically the most diverse cell type. Large cross-species diversity may also be true in sperm physiology and metabolism but surprisingly few data exist. Here we summarise the current knowledge of metabolic pathways of insect sperm, and of parameters and methods used to characterise sperm metabolism. We also use metabolic mapping methods such as NADH fluorescence lifetime decay (FLIM) and H_2O_2 screening (mitoB/mitoP ratio) and present empirical data on several aspects of sperm metabolism. Specifically, we characterise between-species variation in sperm cell metabolic rate and sperm ROS production rate in two insect genera, Drosophila and Cimex.

Keywords: Sperm physiology, sperm metabolism, reactive oxygen species, Drosophila, bedbug

PO520

MODULATORS OF E75 NUCLEAR RECEPTOR ACTIVITY AFFECT THE ACCUMULATION OF TRIACYLGLYCEROL IN LARVAE OF THE BEETLE *TRIBOLIUM CASTANEUM*

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Obesity is a global pandemic that causes significant health expenses due to its comorbidities, such as type II diabetes, hypertension, and atherosclerosis. However, the drugs available for the treatment of obesity are inefficient, or have serious adverse effects, or are expensive, reducing the range of patients under treatment. In this way, the discovery of new molecular targets for the development of new drugs is urgent. For this, we are using the *Tribolium castaneum* beetle as a model of lipid metabolism, obesity and diabetes, exploring the modulation of E75 nuclear receptor activity. Gases, such as CO and NO, bind to the heme group of E75, altering its transcriptional activity. Treatment of *T. castaneum* larvae with SNAP (a NO donor) caused a reduction in the amount of triacylglycerol in the larvae, whereas treatment with CORM-2 (a CO donor) caused an increase in this accumulation. Both treatments were unable to alter the fatty acid oxidation capacity of the larvae. Treatment with CORM-2 also did not alter the *de novo* fatty acids synthesis capacity of the insects. However, treatment with SNAP induces an increase in expression of the fatty acid synthase gene. Finally, treatment with L-NAME, an inhibitor of the endogenous synthesis of NO, did not alter the accumulation of triacylglycerol. These results indicate that both NO and CO modulate the lipid metabolism of *T. castaneum* larvae, although the mechanism is still unclear. We will investigate if there is any modulation in food consumption that could explain the differences in triacylglycerol amounts. We will also investigate the involvement of E75 with the use of RNAi in the future.

Keywords: Lipid metabolism, Fatty acid oxidation, Triacylglycerol, Fatty acid synthesis, qPCR

PO521

ISOLATION AND CHARACTERIZATION OF ALPHA-(1→3)-GLUCAN-DEGRADING BACTERIA FROM THE GUT OF *DIAPERIS BOLETI* FEEDING ON *LAETIPORUS SULPHUREUS*

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Fungivorous insects require special digestive capabilities to take advantage of the high nutritional value of fungal tissue. The compact hyphal tissue of large fruiting bodies of polypore fungi is, in this regard, a particularly demanding environment. The cell wall of fungi is a rich source of alpha-(1→3)-glucan. Especially in the cell wall of mature fruiting bodies of a polypore fungus *Laetiporus sulphureus*, the glucan content was much higher than in other mushrooms, reaching up to 78% of dry matter. The fruiting bodies of this fungus are often populated by insects, which can quickly and near completely disintegrate the colonized mushroom Bacteria degrading alpha-(1→3)-glucan were sought in the gut of fungivorous insects feeding on fruiting bodies of *L. sulphureus*. One such isolate, from *Diaperis boleti*, was selected in an enrichment culture in the glucan-containing medium. The bacterium was identified as *Paenibacillus* sp. based on the results of the ribosomal DNA analysis. The *Paenibacillus* exhibited enzyme activity of 4.97 mU/cm^3 and effectively degraded fungal alpha-(1→3)-glucan, releasing nigerooligosaccharides and a trace amount of glucose. This strain is the first reported alpha-(1→3)-glucan-degrading microorganism in the gut microbiome of insects inhabiting fruiting bodies of polypore fungi.

Keywords: *Diaperis boleti*, fungivore, alpha-(1→3)-glucanase, *Paenibacillus* sp.

Session 14.
Social Insects and Apidology

Symbiosis in Social Insects - Behavioural and Ecological Interactions

PO522

Poster moved.

PO523

Poster moved.

PO524

Poster moved.

PO525

Poster moved.

PO525a

A MULTIFACTORIAL APPROACH OF STRESS FACTORS INFLUENCING BEE HEALTH

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Since the late '90s, a complex pathology known as Colony Collapse Disorder (CCD) brought to widespread events of honeybee disappearance. Many adversities may be responsible of honeybee decline: recrudescence of old and new pathologies, contamination from pesticides and emerging contaminants (*e.g.* nanoparticles) and environmental stresses, including climate change. Stress factors may interact among them with additive or synergic effects and, currently, a multi-stress condition is accepted as the most probable and diffuse decline cause for honeybees. This work is an in-field evaluation of combined stress factors (pesticide mixtures, electromagnetic fields, pest and diseases) on honeybees. Experimental hives were placed in April 2017 in a "control site" far from agricultural field and from significant human settlements and in an "exposure site" located inside an experimental farm where both a high-voltage electric line is present and multiple chemicals treatments for orchards crops are applied. Honeybee families were sampled weekly throughout the year to evaluate health status (parasites, virus, and bacteria) and population parameters (queen presence, brood status, mortality in underbaskets and honey and pollen reserves) and monthly for biomarker analyses (acetylcholinesterase, catalase, glutathione S-transferase, alkaline phosphatase, reactive oxygen species (ROS), lipid peroxidation, and DNA fragmentation). Preliminary data analysis revealed that population parameters and biomarkers were both affected by stress sources. Direct effects on bees were registered in relation to pesticide applications. Multi-stress position showed the most severe effects, leading to colony death. Colony losses were directly related to the development of different pathologies. Reduced immunity defences and social disorders by frequent queen replacement seem to be the main effects related to the multi-stress condition.

Keywords: *Apis mellifera*, colony collapse disorder, desease, biomarker

Bees: Management, Ecosystem Services, and Products

PO526

REARING WILD BUMBLEBEE COLONIES IN THE LAB: CATCHING AND MANAGEMENT TECHNIQUES FOR WILD-CAUGHT *BOMBUS TERRESTRIS* QUEENS

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Bumblebees are key pollinators, at a global scale, and have also become a model organism to address questions in the fields of evolutionary ecology, host-parasite systems, and inclusive fitness. While commercially-produced bumblebees are often taken advantage of by researchers, these questions are increasingly being addressed through the practice of wild bee catching and colony rearing. Such colonies better represent wild and locally adapted populations, and provide a better understanding of the full life cycle of the species, from laying queen through colony growth to sexual reproduction. However, in contrast to work in commercial bumblebees, little is known about how to maximise colony-rearing success with wild-caught queens. Here, we analyse two years' worth of data from wild *Bombus terrestris* queens, from catching to colony establishment. We ask how catch date, and the parasite *Crithidia bombi*, impact the success of colony establishment under laboratory conditions. Our results will help to advise the field with valuable information on optimal bumblebee catching and rearing techniques.

Keywords: *Bombus terrestris*, bee rearing, wild colony, parasite, *Crithidia bombi*

PO527

HONEY BEE-COLLECTED POLLEN USED TO REAR BUMBLEBEES IS A SOURCE OF PATHOGENS AND CAN CAUSE *ASCOSPHAERA APIS* INFECTION IN BUMBLEBEE LARVAE

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Bumblebee trade started in the early ninety's mainly for pollination purposes of greenhouse crops. The market has grown ever since, expanding from high value crops to several fruits and vegetable crops. Managed bees' heath has been in the spotlight, because pathogen spill over from managed bees toward wild bees is possible. Pathogen spill over from managed bees is a threat for the dynamic equilibrium of natural host-parasite interactions and should be avoided. Ensuring pathogen-free bumblebee rearing is a first requisite. The major pathogen influx route in bumblebee breeding facilities is the main protein source, honey bee-collected pollen. The aim of this study was to perform a large pathogen screening on non-irradiated honeybee-collected and to investigate its infectivity-risk to *Bombus terrestris*. A total of seventeen different pollen batches from several different suppliers were analyzed with molecular detection techniques using different sets of broad range and species-specific primers. We identified five different parasites (*Apicystis bombi*, *Ascosphaera apis*, *Crithidia mellificae*, *Nosema* spp. and *Paenibacillus larvae*) and four viruses (CBPV, DWV, IAPV and SBV). An infectivity test revealed that A. apis was able to cause true infections, confirmed by qPCR and observation of typical symptoms. We present first evidence of A. apis infected bumblebee larvae, a parasite first described in the honey bee. Breeding facilities needs to be caution and new diagnose studies in managed bees and control method are crucial.

Keywords: *Ascosphaera apis*, bumblebee, managed bee, pathogens, pollen

PO528

MULTI-DIMENSIONAL MODELLING TOOLS FOR THE ASSESSMENT OF HONEY BEE COLONY HEALTH, PRODUCTIVITY AND POLLINATION SERVICES

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Honey bees are fundamental for the provision of goods (*e.g.* honey, beebread, royal jelly etc.) and ecosystem services (*e.g.* pollination) considered important for the maintenance of biodiversity and food security. Furthermore, beekeeping activity generates employment and represents a major source of income in many rural areas. Honey bee health is highly influenced by environmental conditions, chemical and biological stressors, beekeepers' management practices, socio-economic conditions and policies adopted for cropping and land use. The multiplicity of factors influencing honey bees and the beekeeping sector makes difficult to implement management strategies aimed at preserving honey bee health while guaranteeing productivity and economic return. There is the growing need to monitor honey bee health and to develop tools able to organize and make available data collected through National and European monitoring projects and initiatives. Furthermore, proper modelling tools are needed to analyze such complex data-sets and provide support for decision-making. We present a methodological framework based on Structural Equation Modelling for large data-sets analysis aimed at assessing honey bee health status and predicting honey bee services provision. Starting from a simulated data-set we developed (i) a Health Status Index (HSI) estimating the influence of abiotic, biotic drivers and beekeeping actions in relation to bee health and (ii) predictive models for the estimation of honey production and pollination services provision considering abiotic, biotic drivers and HSI. The results provided information on the relative importance of the main driving variables on honey bee colony health, honey production and provision of pollination services. The proposed methodology can be used for the holistic assessment of honey bee health and productivity and to support decision-making for relevant stakeholders (beekeepers, risk assessors, policy-makers etc.) at local, regional, national and European level.

Keywords: Honey bees, beekeeping, Health Status Index, predictive model, decision support

PO529

BOTANICAL GARDENS AS IMPORTANT REFUGES FOR POLLINATORS TO FACE HABITAT LOSS

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The importance of green areas in the cities, both for people and for the ecological services they sustain, has recently received an increasing attention. In these urban contexts, botanical gardens represent a separate, peculiar category. Actually, what distinguishes these areas is the fact that the preserved plants are often exotic and that, in the case of Angiosperms, there is a wide variability of flower anthesis and morphology in a restricted area. We should also consider that there is an increasing worry about dramatic pollinator's losses, but that little investigation has pointed out botanical garden potential as shelter and food source in urban depleted areas. During the summer 2016 and 2017 at the Ghirardi's botanical garden on the west coast of Garda Lake (Italy), we performed a multidisciplinary survey to understand the actual networks established between exotic flowers and native pollinators. We provided an accurate check-list of the preserved plant species with information on their flowering stage and intrinsic characteristics, and listed the Hymenoptera pollinators visiting them. Plants were grouped according to the flower morphology and to the resource (pollen, nectar) they may collect, as far as it is known. Pollinators were grouped on the base of functional groups (small bees, medium bees, large bees). Interaction webs were then obtained. The strength of this research lies within the topics touched by the discussion that arises from our results: botanical gardens as resource-rich environments for threaten pollinators.

Keywords: Wild bees, plant-insect interaction, ecological niches

PO530

INHIBITION OF *PAENIBACILLUS LARVAE* BY FOODBORNE AND RESIDENT LACTIC ACID BACTERIA

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The loss of bee hives and honey bees caused by parasites and pathogens affecting their colonies has historically represented a major concern, which lead to the continuous search for methods and active substances limiting their deleterious action. A relatively new and promising field of research targets bee intestinal microbiota as a source of bacteria with potential against microbial infections. We have preliminarily evaluated the *in vitro* inhibitory effects of lactic acid bacteria (LAB) in the genera Lactobacillus and Enterococcus isolated from either fermented food matrices or honey bee gut against *Paenibacillus larvae*, the causative agent of American Foulbrood (AFB). These LAB strains were identified by 16S rRNA gene sequence analysis. The screening of around 100 LAB isolates revealed a general antimicrobial activity against *P. larvae*. Experiments were conducted employing inhibition assays on BHI soft agar plates where the growth of *P. larvae* in contact with LAB culture suspensions was monitored during incubation, so as to detect and measure the formation of inhibitory halos within the next 48 h. As a result, several bacterial isolates showed a significant antagonistic effect on the bee pathogen. These results are in line with previous studies and support the generally recognised beneficial properties of LAB for honey bee health. In addition, we have selected specific bacterial strains with higher potential against *P. larvae*. Future studies will focus on the mechanisms through which these strains act against the bee pathogen and on the molecules involved in such interaction. The research program will also involve *in vivo* assays aiming at evaluating the possible beneficial effects of LAB on the whole bee colony.

Keywords: Honey bee pathogen, probiotics, LAB, American Foulbrood

PO531

OBSERVATIONS ON POLLINATING INSECTS IN DIFFERENT ECOSYSTEMS (SARDINIA, ITALY)

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The decline of pollinating insects in the industrialized areas jeopardizes seriously the conservation of many spontaneous and cultivated plant species. In natural and agro-ecosystems, the beneficial effects deriving from their activity are essential to ensure plant reproduction and quantitative-qualitative improvement of several crop productivity. The knowledge on biodiversity and bio-ethology of these insects represents a major aspect to understand the impact of adverse factors on their survival and dynamics in the environment. In the last thirty years, several observations on pollinators in different ecosystems were carried out in Sardinia (Italy). A considerable richness of species (about 180) belonging to Apoidea group was observed. Moreover, rare and infrequent bees were sampled, included endemic entities. The purpose of this long-term overview is to delineate the diversity and abundance of the pollinating insects under different environmental conditions in a Mediterranean region, that helps to realize a regional checklist and contributes to the knowledge of population trends of the endangered species. Another purpose of this work is to evaluate the incidence of solitary bees compared with social species, particularly honeybees and bumblebees, in different agro-ecosystems.

Keywords: Social bees, solitary bees, pollinating service, endemic species

PO532

THE STATUS OF HONEY BEE HEALTH IN ITALY: RESULTS FROM THE NATIONWIDE MONITORING NETWORK

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In the last decades several bee mortalities and colony losses have been reporting in North America and Europe. In response to significant honey bee colony mortality reported in Northern Italy in 2008, a ban on neonicotinoid-dressed maize seeds and a nationwide monitoring network have been established to identify the main factors involved in honey bee decline. The Italian nationwide monitoring network has been effective for 5 years in the framework of two national projects: ApeNet (2009-2010) and BeeNet (2012-2014) funded by the Italian Ministry of Agriculture. The ApeNet and BeeNet networks involved more than 100 and 300 apiaries (10 hives each), respectively, distributed in all Italian regions. Colony and environment data were collected four times per year: after winter, in spring, summer and late autumn. Overall, we found annual and regional variations in pathogens responsible for infection (DWV, ABPV, CBPV, *Nosema ceranae*), Varroa prevalence, level of protein and pesticide contaminations of bee-bread. However, most of these parameters showed clear seasonal trends: we found high prevalence of CBPV and *N. ceranae* in spring while DWV and ABPV were more prevalent in autumn. We also found a significant seasonal variation in the quantity of protein (% raw protein) in bee-bread which was higher in spring (~23%) than in summer (~21%). Bee-bread was often contaminated with at least one pesticide (from 27 to 56%) and the number of detected pesticides was positively related to the extension of the agricultural area surrounding the apiaries. During the 5-year monitoring study, annual colony losses fluctuated over the years from 8 to 19%, with remarkably lower values compared with the data of previous years (2007-2008: from 20 to 38% based on COLOSS questionnaire). Our results emphasize the need to integrate abiotic and biotic factors in a holistic approach in order to define the honey bee health status.

Keywords: Honey bee health, monitoring, Italy

PO533

HARMONIC RADAR TRACKS *VESPA VELUTINA* FLIGHT TO NESTS

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Harmonic radars have been used to track the flight of various insect species. Within the LIFE14 NAT/IT/001128 STOPVESPA project - Spatial containment of *Vespa velutina* in Italy and establishment of an Early Warning and Rapid Response System - a harmonic radar prototype has been developed for tracking the invasive hornet *Vespa velutina*. The radar is characterized by a vertical polarization of the radiated field and advanced

processing techniques able to suppress clutter and improve target detection. The radar is capable to cover 360° in the horizontal plane and a large field of view in the vertical plane (20°). It allows to follow the tracks of the hornets tagged with a 12.3 mm wire antenna and a diode (12.1 mg) up to 470 m. The harmonic radar has been used in autumn 2017 in the westernmost part of Liguria (Italy), where *V. velutina* is present since 2012, and allowed the detection of three nests that were immediately destroyed. The radar will be used in 2018 for the control of *V. velutina* diffusion in Italy, but it could find use in several other fields of entomological research and pest management.

Keywords: *Vespa velutina*, harmonic radar, nest, insect tracking, Asian hornet

PO534

STUDIES ON THE USE OF HONEYBEES (*APIS MELLIFERA* L.) AS BIOINDICATORS OF SOME ENVIRONMENTAL CONTAMINANTS

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Since 1935 honeybees have been used as bioindicators as they actively "visit" all the environmental sectors (vegetation, air, water, soil) of a large area around the hive. In our study, the content of lead, chromium, nickel, benzo[a]pyrene, chlorides, phosphates, nitrates and sulphides was assessed in honeybee- and honey samples collected in two apiaries located in an urban and a suburban area of Bologna (Italy). The aim was to determine the differences in the honeybee contamination levels between: a) exiting *vs* returning foragers; b) morning *vs* afternoon sampling time; c) forager's body surface *vs* inside; d) urban *vs* suburban apiary. The results showed that higher concentrations of heavy metals were found in the bee body surface, in returning foragers, in bees collected in the afternoon and in the urban apiary. Benzo[a]pyrene was only found in honeybee samples collected in urban apiary and, in most cases, higher values were registered in the afternoon compared to the morning. Unexpectedly, higher levels of lead, chromium and nickel were found in the honey collected in the suburban compared to the urban apiary, while benzo[a]pyrene concentrations were always below the LOQ. The levels of chlorides, nitrates, sulphates and phosphates were higher in the internal than in the external part of bee body. Overall, the contamination levels of these pollutants were similar between the urban and the suburban apiary, except for the nitrates contamination in honey that was significantly higher in the urban area. In conclusion, we showed that several parameters can affect the contamination levels in honeybees and they should be considered when using bees as bioindicators of environmental pollution.

Keywords: Bioindicators, honeybees, pollution

PO535

BIOLOGICAL INVASION OF THE DWARF HONEY BEES, *APIS FLOREA* INTO N AFRICA

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A. florea is one of the major honeybee specoce distributed maily in Asia. It was known from some parts of Africa in Sudan and Ethiopia. It was recorded for the first time in Suez, Eastern part Egypt in 2015. The bees were collected during several research expeditions started in late 2015 till now. The colonies of bees were recorded at different areas of the Suez Governorate. The workers were recorded visiting several floral resources such as baslic, citrus and *Tamarix* sp. The tracking of the species movements indicated that the species is moving towards other localities Al Ganian "15km from Suez" towards Ismailia road, 10th of Ramdana City "Cairo – Ismailia road" and Alexandria. So probably in the next years the bees might invade other countries of northern Africa.

Keywords: *Apis florea*, invasion, dwarf honeybee, Suez, Egypt,

Social Immunity

PO536

Poster moved.

PO536a

WHEN PREDATOR ODOR MAKES GROUPS STRONGER: EFFECTS ON BEHAVIORAL AND CHEMICAL ADAPTATIONS IN TWO TERMITE SPECIES

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Predation is a key process in evolution because it impacts directly individual survivorship. Being able to detect a predator before any direct contact is therefore primordial to improve prey's fitness. In insects, chemical communication is involved in several interactions like courtship behavior, circadian activity, parasitism and of course prey-predator interactions. Among the active chemical signals, the cuticular hydrocarbons are known to rule the social organization of social insects including their caste and recognition systems. In this study, we asked if the cuticular hydrocarbons produced by a predator could influence the social organization of its prey. Indeed, hydrocarbons are sticky and could be released in the environment giving indirect information about predators (or competitors). Preys could therefore adapt their behavior which could induce social-organization modifications of their colonies. To test this hypothesis, we studied interactions between two social insects, the ant predator and its termite prey. Two termite species among the most widespread species in Europe were chosen: *Reticulitermes flavipes* and *R. grassei*. Our results show that, under the influence of the predator odor, *R. grassei* presented a higher homogenous chemical profile and also increased its survivorship during competition tests. Interestingly, the predator-odor effects on the behavioral and chemical adaptations of the native species *R. grassei* are not effective in the invasive termite *R. flavipes*.

Keywords: Aggressive behavior; cuticular compounds; competitive tests; *Reticulitermes grassei*; *Reticulitermes flavipes*; subterranean termite

PO536b

MOLECULAR ANALYSIS OF THE EXTENT OF THE *PARACLETUS CIMICIFORMIS-TETRAMORIUM* INTERACTION

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The aphid *Paracletus cimiciformis* (Fordini: Eriosomatinae) was recently shown to exhibit an unprecedented interaction with ants of the genus *Tetramorium*. During the root-dwelling phase of this aphid species life-cycle, two alternative, very distinct wingless morphs are produced that show completely different interactions with *Tetramorium* semilaeve ants. The green (or round) morph shows a typical trophobiotic interaction with the ants (similar to that exhibited by other species of Fordini), in which the aphids excrete sugar rich honeydew that ants consume. In return, ants provide aphids with their care and hygienic services. However, the white (or flat) morph by apparently mimicking the ants'larvae odour, is transported by the ants to their nests where the aphids suck the ants' larvae haemolymph. This was the first reported case of aggressive mimicry in aphids. Although this interaction was originally described between *P. cimiciformis* and *T. semilaeve*, other species of genus *Tetramorium* were shown to interact with this aphid species and other members of the Fordini tribe (specially of the genus *Geoica*) seem to interact with this ant genus. Although the taxonomy of Fordini is starting to be elucidated, the complex taxonomy, and phylogenetic relationships among species of genus *Tetramorium* ants is still far from clear. In the context of studies aimed at characterising the extent of aggressive mimicry in *P. cimiciformis*, we used two molecular markers (mitochondrial COI and nuclear EF1alpha) to stablish the species/lineages of aphids associated to particular species of *Tetramorium* ants in the Iberian Peninsula and how this could be related with the aggressive mimicry described in *P. cimiciformis*.

Keywords: Aggressive mimicry, symbiosis, phenotypic plasticity, aphids, ants

PO536c

FEEDING STRATEGIES AND INTRASPECIFIC COMPETITION IN *VESPULA GERMANICA* IN AN APIARY CONTEXT

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German yellowjacket (*Vespula germanica*) is an opportunist predator and a scavenger, whose eclectic diet includes also honey, brood, dead and live honey-bees. For this species there is no evidence of coordinated attacks involving other conspecifics, while, intraspecific competition has been observed between two or more individuals after predation and during necrophagy. The aim of the present study was to gain further knowledge on feeding behaviour of *V. germanica* to evaluate its role in an apiary context. For this purpose, sight observation on predation and necrophagy behaviours were carried out at the ground level near hives. Furthermore, the degree of dismemberment and the specific body part sequestered by wasp were also reported. Finally, we investigated how intraspecific competition processes can influence the feeding display in this species. Our results confirm a major role of *V. germanica* as scavenger, because its diet is based mostly on bee carrions. It also shows a general preference for thorax, being the body section with the highest protein degree. The intraspecific competition during feeding is very frequent. Despite this, only in 3 cases the interference of another wasp caused the failure of the attack and bee escape. Our study also revealed that intraspecific competition events increase when resource is fresh (predation *vs* necrophagy) and that the number of competing wasps was significantly higher when food was represented by pupae and drones, compared to adult bees. When competition involved two individuals (the most frequent case), the winner was more frequently the first wasp that reached the resource in both predation and necrophagy events. This result suggests that the energy invested in foraging or predating activity is usually rewarded.

Keywords: German yellowjacket, European wasp, predation, necrophagy, agonistic interactions

PO536d

A PRELIMINARY STUDY OF NEW TECHNOLOGY FOR BEEHIVES MONITORING

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The honeybee, *Apis mellifera*, is unquestionably considered an important and significant contributor among the animal pollinators, playing an essential role in the prosperity of the world's ecosystems and indeed to life itself. The aim of this work was to study a procedure that could integrate visual observations carried out on the families and measurable parameters such as sound, temperature, humidity, CO_2 and hive weight, in order to detect information of the state of the hive. The parameters were acquired with sensors placed in the hive. The system was installed in a colony composed by three bee hives positioned within the University Campus. Bees families were evaluated once a week, looking at morpho-productive characters: queen´s prolificacy, brood´s capacity, family strength, brood´s viability, increased amount of wax combs in the nest, the quantity of honey and bee bread accumulated in the nest. An hardware platform and a software system to acquire and analyze data from the sensors supports the analysis of the data obtained from the observations. In this context, the proposed work aims at studying and developing an innovative system capable of giving to the beekeepers an early analysis of beehives condition, detecting anomalous situations and predicting particular event. In this work, preliminary results will be reported.

This study was financially supported by the Università Politecnica delle Marche, Ancona, Italy, within the project "NuHive: New technology for beehives monitoring".

Session 15.
Urban Entomology and Stored Product Protection

Integrated Pest Management in Stored Product Protection

PO537

FUNGI FOR CONTROL OF STORED PRODUCTS INSECT PESTS

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Pests of stored cereal products can be controlled with pesticides, but there is a risk of residues affecting human health, and the pests frequently become resistant. The food industry urgently needs alternative approaches for control of stored product insects, and entomopathogenic fungi are one possible control agent. This project aims to investigate commercially available fungi to assess their effect on the insects. The mechanisms of infection, the impact on insect behaviour and the prospects for effective control will be investigated.

Keywords: Entomopathogenic fungi, Pests of stored

PO538

REPELLENT ACTIVITY OF THREE *HUMULUS LUPULUS* (L.) ESSENTIAL OIL TERPENES AGAINST *SITOPHILUS GRANARIUS* (L.)

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The essential oil (EO) obtained by hydro-distillation of hop (*Humulus lupulus*, L.) clusters has been shown to possess a strong repellent effect against the granary weevil *Sitophilus granarius* (L.) (Coleoptera, Curculionidae). In the present study, gas chromatography-mass spectrometry analysis showed that ß-caryophyllene (12,6%), farnesene (9,5%), and ß-myrcene (6,6%) are among the main components of hope cluster EO. A two-choice pitfall bioassay was used to assess the behavioural response of adult granary weevils to ascending doses (0.5 to 4.5 mg) of each of these terpenes either alone or in the presence of wheat grain (200 g) odours. The test arena was a steel container (32 cm diameterx7 cm height) with two diametrically opposed holes (3 cm diameter) on the bottom and two glass flasks (500 ml), assigned to collect insects, positioned under each hole. In the dose range tested, all compounds elicited negative mean response index (RI). The mean RI ranged from -0.02 to -42.50 for ß-myrcene, from -5.23 to -36.48 for farnesene, and from -5.83 to -30.84 for ß-caryophyllene. In the presence of increasing doses of each terpene, the mean RI to wheat grain odours (79.84) was significantly reduced and ranged from 4.17 to -23.33 for ß-myrcene, from -2.50 to - 30.84 for farnesene, and from 0.00 to -28.67 for ß-caryophyllene. In these latter experiments, the negative RI values induced by the highest doses of test compounds demonstrated their capability to effectively disrupt granary weevil orientation to the host substrate and suggested their role in determining the strong repellent effect of hop cluster EO against *S. granarius* adults.

Keywords: Storage pests, botanicals, behavioural bioassays, repellents

PO539

SWEET ORANGE ESSENTIAL OIL NANOFORMULATION APPLIED AS COLD AEROSOL AGAINST *TRIBOLIUM CONFUSUM* AND *CRYPTOLESTES FERRUGINEUS*

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Common control strategies to manage stored product pests are mainly based on the use of synthetic insecticides for direct applications and as fumigants. The use of chemical pesticides in food industry is strictly regulated, limiting the range of available products. However, the rising demand of consumers for pesticide-free food and the increasing resistance of pests to traditional insecticides, dictate the need to evaluate alternative control methods and to develop new eco-compatible insecticides. For this purpose, many sustainable techniques have been tested for the control of stored product pests. Plant essential oils (EOs) have been extensively studied due to their acknowledged antimicrobial, insecticidal, antioxidant and antifungal activity, and Citrus essential oils can represent a valid alternative to synthetic insecticides. An oil in water nano-emulsion of Sweet Orange EO has been formulated with the aim to maximize its efficacy as insecticide against two stored product pests. The developed insecticide, applied as cold aerosol, was evaluated against adults of *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) and *Cryptolestes ferrugineus* Stephens (Coleoptera: Cucujidae). Both chemical and physical characterization of the EO-based formulation was carried out. The average size of the developed formulation belonged to the nanometer scale (average size 230.3 nm), with low polydispersity index (Pdi 0.274). The stability over time of the tested formulation was confirmed by the relatively high zeta potential value obtained (ζ= 26.93). The efficacy of the tested formulation showed a dose-dependent response and the cumulated mortality of the exposed insects increased until 24h of exposure. *Tribolium confusum* proved less susceptible to the tested formulation (LC50=86.30 ppm; LC99 631.14 ppm) than C ferrugineus (LC50=36.79 ppm; LD99=209.7 ppm). The results obtained in these trials, together with the large availability at reasonable costs of Sweet orange EO, are promising for the potential development of new tools to control stored product pests.

Keywords: Stored product pests, Botanicals, sustainable control methods

PO540

PRELIMINARY OBSERVATIONS ON THE SUSCEPTIBILITY OF PSEUDO-CEREALS AND OTHER NEW GRAINS FOR THE EUROPEAN MARKET TO THE ATTACK OF STORED PRODUCT INSECTS

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Until a few years ago, pseudo-cereals and some other plants (originating either from Latin America or from Asia or Africa) of interest as food were almost unknown in Europe, but recently they have attracted the attention of researchers because of their growing diffusion in the agri-food industry. In the human diet, pseudo-cereals such as quinoa (*Chenopodium quinoa* Willdenow), amaranth (*Amaranthus* spp.) and buckwheat (*Fagopyrum esculentum* Moench) are appreciated for some properties including high nutritional values; similarly chia (*Salvia hispanica* L.), which is an oily seed, and teff (*Eragrostis tef* (Zuccagni) Trotter), which is instead a cereal, have interesting nutritional profiles. The grains of these plants can be consumed in the place of traditional cereals, as such or transformed into flour to obtain bread, pasta or other derivatives. Their cultivation is increasing in various countries of Europe, including Italy and, as for all (other) cereals, they are susceptible to attack by pests after harvest. This aspect cannot be ignored. Our laboratory research has investigated the susceptibility of pseudocereals, chia and teff to attack by some common stored-product beetles and moths known for their more or less marked polyphagy: *Rhyzopertha dominica* (F.) (Bostrichidae), *Sitophilus granarius* (L.) (Dryophthoridae), *Tenebrio molitor* (L.) (Tenebrionidae) and *Plodia interpunctella* (Hübner) (Pyralidae). Results revealed significant differences in the timescale and means of infestation and larval development: *S. granarius* and *R. dominica* grew only on buckwheat; *P. interpunctella* grew on all seeds except amaranth, with high larval trophic activity, but most larvae died between 60 and 90 days after the beginning of the infestation. *T. molitor* larval activity was observed on all the grains and the life cycle was not interrupted. This study, still underway, is a first step towards optimizing the storage methods of these new products and to understanding their main risks of infestation and contamination.

Keywords: quinoa, amaranth, buckwheat, chia, teff, post-harvest, beetles, moths, damage

PO541

HOST PREFERENCE AND EFFECT OF DIFFERENT TEMP & RELATIVE HUMIDITY ON WEIGHT LOSS AND PERCENT INFESTATION IN DIFFERENT STORED GRAINS BY *CALLOSOBRUCHUS MACULATUS*

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To study the host preference and to determine the best humidity and temperature for grain storage pest *C. maculates* the present research work was conducted at the Research Laboratory of Department of Entomology, The University of Agriculture Peshawar during the year 2012. Three sets of the experiment were conducted at 20°C and 65% R.H, 30°C and 75% R.H at room temperatures. The experiments were carried out in randomized complete block design comprising of five treatments (Kidney bean, mung bean, gram, chickpea and wheat) with three replications. Results showed that the most preferred food of *C. maculates* was kidney bean and mung bean whereas the least preferred food of *C. maculates* was gram, chickpea and wheat. The percent infestation recorded at 20+2°C and 65+5% R.H. was more in wheat i.e 4.60% followed by gram, kidney bean, mung bean and wheat which recorded percent infestation of 3.79, 3.16, 3.00 and 1.04%, respectively. At 30+2°C and 75+5% R.H. The highest (23.75%) percent infestation was recorded in kidney bean followed by mung bean, wheat gram and chickpea i.e 11.51, 7.43, 4.61 and 1.50 respectively. Results further showed that at room temperature the percent infestation was highest (7.62, 6.63%) in kidney bean and mung bean as compared to the chickpea where the percent infestation was recorded lowest *i.e.* 1.15. In conclusion of the present study revealed that the most preferred food of *C. maculates* was kidney bean and mung and highest percent infestation was recorded at 30+2°C and 75+5% R.H whereas the lowest was recorded at 20+2°C and65+5% R.H. The study recommends that grains should be stored at 20-25°C and 70-75% R.H to reduce the losses in grains.

Keywords: *C. maculates*, Humidity, Temperature, Stored grains.

PO542

COMPETITION BETWEEN *ANISOPTEROMALUS CALANDRAE* (HOWARD) AND *THEOCOLAX ELEGANS* (WESTWOOD) (HYMENOPTERA: PTEROMALIDAE), LARVAL PARASITOIDS OF THREE HOST SPECIES OF STORED-PRODUCT INSECT PESTS

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The two pteromalids, *Anisopteromalus calandrae* (Howard) and *Theocolax elegans* (Westwood) are solitary larval ectoparasitoids to suppress many stored-product insect pests. The less knowledge competitive of two pteromalid parasitoids on different hosts was limited than other pteromalid parasitoids. We investigated the competition between two pteromalids *A. calandrae* and *T. elegans* on three host species of Coleoptera: Lesser Grain Borer, *Rhyzopertha dominica* (Fabricius) (Bostrichidae), Maize Weevil, *Sitophilus zeamais* Motschulsky (Curculionidae) and Cowpea Weevil, *Callosobruchus maculatus* (Fabricius) (Chrysomelidae) under laboratory condition. *Anisopteromalus calandrae* and *T. elegans* was released 2, 6, 12, 20 and 30 pairs to parasitize host larvae of 23 days-old *R. dominica*, 21 days-old *S. zeamais* and 14 days-old *C. maculatus. Rhyzopertha dominica, S. zeamais* and *C. maculatus* developed on wheat grain, brown rice and cowpea bean, respectively. Single species and combined species were used for experiments. Neonate parasitoids were mated for 24 hours. Female parasitoids were released to parasitize for two days and taken out. Number of *A. calandrae* progeny emerged from *S. zeamais* larvae higher than *T. elegans* progeny. Both pteromalid parasitoids progeny produced on *S. zeamais* larvae were highest. We found *A. calandrae* and *T. elegans* were low parasitism on *C. maculatus* larvae. The insectary production of *A. calandrae* and *T. elegans* was discussed.

Keywords: *Anisopteromalus calandrae*, Biological control, competition, *Theocolax elegans*

PO543

IDENTIFICATION AND QUANTIFICATION OF PHYTOECDYSTEROIDS AND CLERODANES FROM AJUGA PLANTS: POTENTIAL ACTIVITY AGAINST THE PEST *SPODOPTERA LITTORALIS*

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The Mediterranean moth *Spodoptera littoralis*, a major crop pest worldwide, is usually controlled by insecticides. Ajuga plants contain specialized metabolites including phytoecdysteroids (analogues of steroid hormones that regulate metamorphoses in arthropods) and clerodanes (diterpenoids exhibiting anti-feedant activities). Our objective is to use these metabolites as environmental friendly control of the moth. Using Liquid Chromatography-Time Of Flight-Mass Spectrometry (LC-Tof-MS) we analyzed the profile of phytoecdysteroids and clerodanes in wild Israeli Ajuga plants (*A. iva*, *A. chamaepitys* and *A. orientalis*) and evaluate their efficiency in reducing the fitness of *S. littoralis*. To test the biological activity of Ajuga extracts, leaves of Castor bean were smeared by a brush with methanol crude extract including phytoecdysteroid and clerodanes (24 and 40gr of dry leaves dissolved in 240 and 160ml MeOH respectively). For each repeat, 10 1st and 3st instar *S. littoralis* larvae were fed with one treated leaf for 3 and 4 days respectively. Larval weight gain (LWG) and survival was compared to control (smeared with water) leaves. Our results demonstrate that the highest contents of the compounds (20-hydroxyecdysone, makisterone A and dihydroajugapitin) were identified in *A. iva* leaves (270, 570 mg grDw-1 and 0.25µg grDw-1 respectively) as compared to *A. chamaepitys* and *A. orientalis* (40.5, 30 mg grDw-1, 0.005 µg grDw-1 and 0.1, 0.3 mg grDw-1, 0.006 µg grDw-1 respectively). Crude extract from *A. iva* leaves (50, 100 and 500µg/µl), significantly increased mortality of *S. littoralis* 1st instar larvae (36%, 70% and 87% respectively) compared to the control (6%). LWG of 3rd *S. littoralis* was significantly decreased (by 65%, 69% and 44% respectively) as compared with the relevant controls. Our results demonstrate the potential use of extraction of *A. iva* as environmentally-safe insect pest control.

Keywords: *Spodoptera littoralis*, Ajuga, phytoecdysteroids, clerodanes and pest control

Urban and Public Health Pests

PO544

COMPLEX OF ARTHROPOD SPECIES THAT DWELL IN HOSPITALS AND THEIR CONTROL

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Disinsection measures were held in the cities of the Moscow region, Moscow, St. Petersburg, Astrakhan. In the premises of different purposes, the following insect species (Insecta) were encountered: lice – body *Pediculus humanus corporis* De Geer, head *Pediculus humanus capitis* De Geer, pubic *Pthirus pubis* (L.), bed bugs *Cimex lectularius* L., cockroaches – German *Blattella germanica* (L.), oriental *Blatta orientalis* L., American *Periplaneta americana* (L.), ants – Pharaoh's *Monomorium pharaonis* (L.), common black *Lasius niger* L., cornfield *L. alienus* (Forster), flies – house *Musca domestica* L., false stable *Muscina stabulans* (Fallen), small house *Fannia canicularis* (L.), blow *Calliphora erythrocephala* (Meigen), brown blow *C. vicina* R.-D., and green blow *Lucilia* ssp., cat fleas *Ctenocephalides felis felis* (Bouche), northern house mosquitoes *Culex pipiens molestus* Forsk., and mites (Acarina): itch *Sarcoptes scabiei* (L.), home dust *Dermatophagoides farinae* Hug., *D. pteronyssinus* (Trouessart), rat *Ornythonyssus bacoti* Hirst, mouse *Liponyssoides sanguineus* (Hirst), chicken *Dermanyssus gallinae* (De Geer). All these species are vectors of pathogens or causative agents of various diseases. In the conditions of medical institutions, they participate in the spread of nosocomial infections. Control of arthropods in hospitals requires compliance with increased requirements to the safety of patients and staff. The standard hospital has a certain set of premises - technical (corridors, cellars, attics, garbage chambers), for personnel (for administration, doctors, nurses, technical personnel), food (kitchens, dining rooms), medical (operating, procedural), patient rooms. In these rooms different arthropods can live. An IPM system has been developed to prevent invasion and settlement, as well as control of arthropods in medical institutions.

Keywords: Disinsection measures, insects, mites, medical institutions

PO545

A NOVEL COMBINATION OF VOCS AND FLOWER-SHAPED TRAP FOR THE CAPTURE OF HOUSE FLY IN DOMESTIC ENVIRONMENTS

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House fly (*Musca domestica* L. - Muscidae), is an insect strictly related to human activities. It can contaminate food storages and utensils by walking, feeding and defecating activities, and transmit more than 100 pathogenic microorganisms. Control strategies for *M. domestica* based on the use of sticky traps are considered a sustainable alternative to synthetic insecticides treatments. Recent studies demonstrated that volatile organic compounds (VOCs) emitted from flowers of myophilous/sapromyophilous plants attract *M. domestica* as pollinator. Similarly, carnivorous plants use a combination of visual and olfactory cues to lure and trap their prey insects. Objective of this study was to evaluate, in domestic environments, the efficacy of an attractant (made by a mixture of several VOCs and produced from GEA Srl – Italy), baited on chromotropic sticky traps mimicking in shape and color the flowers of carnivorous plants belonging to the genus Dionea. The behavioral bioassays (two-choice experiments) were carried out simultaneously in two different rooms of 16 (room A) and 20 m^2 (room B) respectively. For each replicate, two flower-shaped traps, one loaded with the attractant (test) while the other unloaded (control) and thirty adults of house fly were used. After 24 hours, the number of individuals captured in test and control traps was counted. The results indicated that in both rooms the traps loaded with the attractant determined a significant increase of captures. The percentage of captured flies in loaded or unloaded traps was 66% *vs* 34% in the room A and 63% *vs* 37% in the room B. These data evidence the possibility to use this novel tool for capturing house flies in domestic environments and underline the importance of the combination of chemical and visual stimuli to optimize their efficacy.

Keywords: *Musca domestica*, attractant, choice experiment, behavioral bioassays

PO546

AN EASY TO APPLY STATISTICAL MODEL TO PREDICT THE DYNAMICS OF *AEDES ALBOPICTUS* AND TO OPTIMIZE THE INTERVENTIONS OF DISINFESTATION IN PUBLIC AREAS IN GENOA

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The Asian Tiger mosquito, *Aedes albopictus* (Skuse), is the most invasive mosquito species in Europe. After the first case of *Chikungunya epidemics*

in Ravenna (Italy) in 2007, since 2009 in Genoa, *Aedes albopictus* is monitored to determine the abundance and distribution of the species in the territory and identify which environmental parameters affect its presence. The aim of our research is to determine which meteorological parameters influence the presence and abundance of eggs and larvae, through a regression model. Unfortunately, we have not a unique model, due to the particular topography of the urban area of Genoa. Its longitudinal extension is about 30 km, and hills and mountains rise steeply in the surroundings. These features determine the presence of different microclimates that may change meteorological parameters locally. Those factors may affect the biological cycle of the Asian tiger mosquito in different ways in three main areas, called 'Centro' (Center), 'Levante' (East) and 'Ponente' (West). For each of them we carried out detailed calculations and statistical analysis by using the standard technique for regression models. So we have individuated three models, simple to apply and depending essentially on temperature, by which we can predict the numbers of of eggs or larvae and their dynamics (for example the period of the year in which hatching and larvae development are more probable) and this is fundamental to support mosquito control activities.

Keywords: *Aedes albopictus*, Statistical Model, Population dynamics, Anova, Seasonal activity, Linear model.

Artifact Pests

PO547

IDENTIFICATION, FIGHT AND PREVENTION OF WOOD, PAPER AND CLOTH PESTS IN ARCHAEOLOGICAL AND HISTORICAL ARTIFACTS

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The problems related to pests, in particular insects, of artifacts of archaeological and historical interest, is topical. In fact, late solutions are often taken only when the weed is revealed. The present study describes both the most innovative techniques that have been developed to early identify the presence of weeds and the latest pest control and protection systems. For each parasite it was necessary to establish an identification technique that could give a screening as soon as possible and without errors. At the same time the best disinfection systems have been developed that are effective while also taking account of the object delicacy. Finally, methods for pest-free sample maintenance have been identified.

Keywords: Woodworm, termite, moth, silverfish, archeology, artifact

Session 16.
Symbiosis and Insect Vector Biology

Molecular and Cellular Interactions Between Insect Vectors and the Pathogens They Transmit

PO548

THIOESTER-CONTAINING PROTEINS IN THE *TSETSE FLY* (GLOSSINA) AND THEIR RESPONSE TO TRYPANOSOME INFECTION

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Thioester-containing proteins (TEPs) are conserved proteins with a role in innate immune immunity. In the present study, we report for the first time a detailed characterization of the TEP family from six *tsetse fly* species (Glossina). Tsetse flies are the biological vectors of several African trypanosomes, which cause devastating disease in in sub-Saharan Africa, sleeping sickness in humans and nagana in livestock. The analysis of the tsetse TEP sequences revealed information about their structure, evolutionary relationships and expression profiles under both normal and trypanosome infection conditions. Phylogenetic analysis of the family showed that *tsetse fly* harbors a genomic expansion of specific TEPs that are not present in other dipterans. We found a constitutive expression of all *TEP* genes in the alimentary tract, mouthparts and salivary glands. *G. morsitans* and *G. palpalis* TEP genes display a tissue-specific expression pattern with some that are markedly up-regulated when the fly is infected with the trypanosome parasite. A different TEP response was observed to infection with *Trypanosoma brucei* compared to *T. congolense*, indicating that the tsetse TEP response is trypanosome-specific. Taken together our findings demonstrate a genomic expansion of *tsetse fly*-specific TEPs and are suggestive for the involvement of the TEP family in tsetse innate immunity with a possible role in the specific control of the trypanosome parasite.
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Keywords: Thioester-containing proteins, *tsetse fly*, trypanosome, innate immunity, vector-parasite interaction

PO549

PRIMARY CELL CULTURES OF IMMUNOCYTES: A NEW TOOL FOR THE *in vitro* STUDY OF INSECT-MICROBE INTERACTIONS

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Several microorganisms inhabit insects' body and exhibit a range of interactions with the host immune system depending on their pathogenic, mutualistic, commensal or saprophytic nature. In particular, when a strict coevolution with the host occurred and lead to a strong genome reduction, some microorganisms are able to escape insect defense responses. It is the case of some insect-borne plant pathogens for which the ability to elicitate or suppress the immune responses has been proved. Moreover immunocytes have been shown to interact with symbionts and play a role in the vectorial capacity of insects. In this scenario, primary cell cultures of phytoplasma vectors' immunocytes have been proposed as a new tool for studying the interactions between the pathogen and the host as well as the interplay between symbionts and immunocytes, in order to better understand insect vector competence. Once the best culture medium has been identified, immunocytes of the psyllid vectors *Cacopsylla melanoneura* and *C. pyri* and of the leafhopper *Euscelidius variegatus* were kept alive for more than 2 months with mitosis activities observed 2 weeks post culture, while adhesion and phagocytosis activities were confirmed by functionality test. *In situ* hybridization revealed that the defensin gene is actively transcribed in cultured *E. variegatus* immunocytes, while cecropins were not recorded in this species. Bacterial challenges revealed the induction of defensine gene only after *Staphilococcus aureus* challenge, but not after *Escherichia coli* and *Asaia* spp. exposure. The possibility to culture insect vector immunocytes and to analyze their ability in synthetize some antimicrobial peptides, opens new opportunities for the study of insect-microbe interactions. In particular, the chance of plant pathogens to induce or modulate the immune responses of the host could provide potential targets for the management of insect vectors in the future.

Keywords: Cacopsylla, *Euscelidius variegatus*, insect vector, defensin

Ecology/Epidemiology/Evolution of the Three-Way Interaction Insect Vector-Pathogen-Host

PO550

ACQUISITION AND INOCULATION OF FD PHYTOPLASMA BY ADULTS OF *SCAPHOIDEUS TITANUS* BALL: LESS TIME IS REQUIRED

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Scaphoideus titanus Ball (Hemiptera: Cicadellidae) is the main vector of grapevine's "Flavescence dorée" (FDP) phytoplasmas (16SrV, subgroups C and D). According to the state-of-art, nymphs acquire phytoplasmas from infected plants within a few days (acquisition access period, AAP), and undergo a latency period (LP) lasting 3-5 weeks. Afterwards the insects, meanwhile having mutate to adults, are able of infecting new plants following and inoculation access period (IAP) of a few days. However, some aspects of FDP epidemics suggest that adults may also acquire directly phytoplasmas and become infective within their lifespan. Therefore, transmission experiments have been carried out under lab conditions, using broad bean plants (*Vicia faba* L.) artificially infected with 16SrV-C phytoplasmas as a source of inoculum. Uninfected *S. titanus* adults were allowed to feed for 7 days (AAP) on infected plants. Afterwards, three inoculations on healthy broad beans were performed at 7 days intervals each (IAPs). At the end of each IAP, insects were moved on a new plant. Therefore, IAP 1, IAP 2 and IAP 3 lasted 7, 14 and 21 days from the end of AAP,

respectively. Phytoplasma were identified in plants and insects by DNA extraction and PCR. Some insects were also dissected and fluorescent *in situ* hybridization was made to detect the presence of phytoplasmas in midguts and salivary glands. Infection in insects and plants ranged 46-68% and 16-23%, respectively. Neither showed significant differences among IAPs, whereas phytoplasma load was significantly higher in IAP3 for both plants and insects. Phytoplasmas were identified in both midgut and salivary glands. The whole transmission process has been therefore successful within just 14 days (AAP=7+IAP=7). These new insights are consistent with severe FDP outbreaks at the end of the summer in grapevine growing areas, and may call for new pest management strategies against *S. titanus*.

Keywords: Flavescence dorée phytoplasma, *Scaphoideus titanus*, Acquisitions by adults, Transmission process

PO551

FIRST ASSESSMENT OF TRANSMISSION RISK OF ARBOVIRUSES IN MOROCCO

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Global changes have induced a recrudescence of vector-borne diseases, with an important change in their distributions. North Africa is as risk of arbovirus emergence, because it links the Mediterranean basin, where exotic vectors, such as *Aedes albopictus*, are spreading leading to dengue or chikungunya outbreaks, and the Sahel known as endemic area for some arboviruses such as Rift Valley fever (RVF), which is an acute viral zoonotic disease affecting both humans and domestic animals, and mostly transmitted by mosquitoes. Morocco should be considered as risk of RVF emergence due to its proximity with Mauritania where the disease is endemic. This work aimed to identify potential vectors of RVF and to characterize the vector role of *Culex pipiens*, a species known to carry this disease. We carried out transversal studies across the whole territory, by collecting adults and larvae aiming to have an estimation of the species abundance. We carried out adults and larvae collection in more than 140 different sites (urban, suburban and rural) during the springs 2015 and 2016, with a special interest for the South of Morocco, considering the proximity of Mauritania. It is the first recent and extensive prospection of Culicidae in this part of the country. The main abundant collected species were: *Culex pipiens*, *Culex theileri*, *Culex simpsoni* and *Culiseta longiareolata*. Aedes species could be underestimated due to the timing of collections. However, we identified for the first time in Morocco the Asian tiger mosquito *Aedes albopictus*. Specimens were identified by both morphology and molecular assay. The population located in Rabat, capital of Morocco, has overwintered during 2015/2016 demonstrating for the first time the establishment of this species in North Africa. We after proceeded at a molecular analysis to characterize the vector role of *Culex pipiens* by looking for all viral communities harbored by this species. This study is still in process to describe the population of pathogenic arboviruses hosted by *Culex pipiens*

Keywords: Vector-borne diseases, emergence, arboviruses, Rift Valley fever, Aedes, Culex

PO552

POTENTIAL AUCHENORRHYNCHA VECTORS OF *XYLELLA FASTIDIOSA* IN CRETE (GREECE): SURVEY, IDENTIFICATION, SEASONAL ABUNDANCE AND VECTOR MANAGEMENT TACTICS

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The "Olive Quick Decline Syndrome" is a serious disease of olives associated with the presence of the bacterium *Xylella fastidiosa*. The pathogen is currently causing major crop losses in Italy and recently in Spain, but it has not been reported in Greece to date. However, the risk of disease expansion is very high for the nearby olive producing countries. For this reason the Region of Crete in collaboration with research institutions and universities is performing actions within the frame of a research program that will be enhancing the readiness of the authorities to eradicate the pathogen when required. A survey of Auchenorrhyncha (Hemiptera), which could potentially act as vectors of *X. fastidiosa*, is carried out in several olive groves of Crete, a major olive growing region of Greece. Adult populations of Auchenorrhyncha species have been monitored fortnightly since June 2017 using Malaise traps and sweeping nets. Initial results indicated high species richness. The most abundant subfamily found was Typhlocibinae followed by Deltocephalinae (Hemiptera: Cicadellidae). Auchenorrhyncha populations were highest from September to December. The species *Philaenus spumarius* and *Neophilaenus campestris* have been recorded in very low numbers. Finally, a wide range of chemical insecticides are currently screened for their efficacy levels on different Auchenorrhyncha species, using a novel laboratory bioassay protocol simulating field exposure pathways. Up to now, more than eight insecticides exhibited mortality levels over 95% at the maximum recommended label rate, suggesting that they could be used as vector control agents within a pathogen eradication scheme. This study offers valuable results for developing rational and efficient strategies against the spread of *X. fastidiosa* as well as important information on species diversity, biology and ecology of the Auchenorrhyncha vectors within the olive-grove environment.

Keywords: *Xylella fastidiosa*, Auchenorrhyncha, Greece, survey, abundance, vector, management, insecticides

PO553

SPATIAL DISTRIBUTION OF PHLOEM-FEEDING SPECIES IN A VINEYARD WITH HIGH INCIDENSE OF BOIS NOIR

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Bois noir (BN) is the most widespread grapevine disease in Europe and Mediterranean area, which can cause severe production losses. BN, associated with ‘Candidatus *Phytoplasma solani*’, is transmitted from herbaceous plants to grapevine by phloem–feeding vectors, mainly by *Hyalesthes obsoletus*. Transmission of pathogens depends on the abundance and dispersal of their vectors, which can be affected by cultivation practices, pedoclimatic conditions and host plant abundance. Dispersal of the phloem-feeding species and their relationship with weed abundances were studied in a vineyard, where a high incidence of BN has been recorded. Adults were sampled (from May to October) using yellow sticky traps (n. 52), placed at a height of 20 cm above the ground in a regular geo-referenced grid, Moreover, a D-vac aspirator was used to collect insects on the vineyard borders. Spatial analysis by distance indices was performed on total insect captures and wild plant species abundances too. The most abundant species collected were: *Neoaliturus fenestratus*, *Thamnotettix zelleri*, *Psammotettix alienus*, *Euscelis lineolatus*, *Laodelphax striatellu* and *Exitianus capicola*. Only 3 specimens of *H. obsoletus* were captured on the traps. The most abundant species collected on the vineyard borders were: *P. alienus*, *E. lineolatus*, *Anaceratagallia laevis*, *N. fenestratus*, *Doratura homophila*, *Recilia schmidtgeni*, *L. striatellus*, *Toya propinqua*, *E. capicola* and *T. zelleri*. Preliminary results of molecular investigations carried out on *A. laevis*, *Anaceratagallia ribauti*, *E. capicola*, *E. lineolatus*, *Mocydia crocea*, *N. fenestratus*, *H. obsoletus*, *Hyalesthes scotti*, *P. alienus*, *Reptalus quinquecostatus* allowed to identify ‘Ca. *Phytoplasma solani*’ in *E. capicola*, *E. lineolatus*, *M. crocea*, *N. fenestratus*, *H. obsoletus*, *H. scotti* and *P. alienus*. Spatial distribution maps and association/dissociation with weeds are reported. The identification of the known and potential vectors of BN phytoplasma associated to vineyard ecosystem is of extremely importance in order to set up effective control strategies.

PO554

THE ROLE OF TRANSOVARIAL TRANSMISSION IN THE MULTITROPHIC INTERACTION BETWEEN *‘CANDIDATUS PHYTOPLASMA MALI’*, ITS INSECT VECTOR *CACOPSYLLA PICTA* AND THE HOST PLANT MALUS×DOMESTICA

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The two phloem-sucking psyllid species *Cacopsylla melanoneura* and *Cacopsylla picta* are well known vectors for the bacterium□*Candidatus Phytoplasma mali* ,□the agent associated with apple proliferation disease. The disease causes severe economic damage in apple orchards in Northern Italy and since there exists no direct treatment against the phytoplasma, a rigorous control of insect vectors is fundamental for limiting the disease spread. Monitoring of the two vector-species *C. melanoneura* and *C. picta* in the last years in South Tyrol revealed a decrease in capture numbers. However, the infection rate of *C. picta* individuals increased over the years. In a recent study, it was shown that *C. picta*, which is the more efficient vector for□Ca. *Phytoplasma mali* □compared to *C. melanoneura*, is able to transmit the bacteria to its progeny. This way of transmission allows the bacteria to bypass the time-consuming reproductive cycle in host plants. In fact, the bacteria exponentially proliferate in the developmental stages of *C. picta*. Furthermore, the vertical transmission leads to an augmented number of infected offsprings and might thus be a reason for the increase in the infection rate of the population over the years. The transovarial transmission has an important impact on orchard management, because it emphasizes the necessity of reducing remigrant and emigrant individuals to avoid oviposition of infected eggs and the fast, exponential reproduction and spread of highly infectious insects. The knowledge about transovarial transmission in *C. picta* provides the basis for a better understanding of the potential spread of apple proliferation disease.

Keywords: Phytoplasma, apple proliferation, epidemiology, insect vectors, transovarial transmission

PO555

DETERMINING THE IMPACT OF INSECT INNATE IMMUNITY ON VIRAL DIVERSITY AND EVOLUTION

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Host-pathogen interactions impose selective pressures that may lead to adaptive evolution on both partners. The Red Queen hypothesis explains these kinds of interactions where species constantly coevolve in winnerless dynamics. To study this defence-counterdefence adaptation circle, we thought to assess the impact of the innate immune system on viral population diversity and evolution using *D. melanogaster* as model insect and its natural pathogen Drosophila C virus (DCV). On one hand, the enormous amount of biological and genetic tools available for the fruit fly, allowed us to generate fly mutants deficient on the main innate immunity pathways, Imd, Toll and RNAi pathways, on the same genetic background. On the other hand, as a consequence of their relative small genomes (aprox. 9,3Kb for DCV), fast replication rates and lack of proofreading activity on their RNA-dependent RNA polymerases, positive RNA viruses, such as DCV, reach high mutation rates and are rapidly evolving pathogens very convenient to use as tools to conduct basic experimental evolution studies. To achieve our goal, populations of 500 flies for each mutant (9 genotypes in total) or wild type flies were fed with DCV or mock fed and virus was serial passaged 10 times. Full-length DCV genome was amplified by RT-PCR after each passage and for each fly genotype, and the viral population diversity was determined by deep sequencing. Results on the impact of the host immune system on viral evolution will be presented.

Keywords: Insect immunity, Drosophila, Virus evolution, RNAi, DCV

PO556

IS HOST SELECTION OF MEDITERRANEAN PINES BY *MONOCHAMUS GALLOPROVINCIALIS* (COLEOPTERA; CERAMBYCIDAE) RELATED WITH PINE WILT DISEASE INCIDENCE?

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The pine sawyer *Monochamus galloprovincialis* is the sole vector in Europe of the pine wood nematode (PWN) *Bursaphelenchus xylophilus*, which has been introduced in Portugal and Spain. The PWN is inoculated by the feeding activity of recently-emerged insects from nematode-infested trees and by oviposition of mature insects. Our objective was to evaluate the selection of pines for feeding and oviposition by *M. galloprovincialis* under semi-natural conditions. On four occasions, adults were released in a closed greenhouse with 400m^3 and allowed to choose between four potted trees ≈10 years old of pine wilt-susceptible hosts (*P. pinaster*, *P. sylvestris*) and of pine wilt-resistant/tolerant hosts (*P. pinea*, *P. halepensis*). For the feeding trial only recently-emerged beetles were used, while for the oviposition trial sexually-mature insects were selected. Volatiles emitted by the pines before and after insect feeding were asserted by solid-phase micro-extraction and gas chromatography-mass spectrometry, and feeding activity was analysed and assessed by digitally measuring feeding areas. For the oviposition, we counted the number of eggs laid on all pines. Insects feed on the four pine species tested, with highest frequency and feeding area for *P. sylvestris* and lowest for *P. pinea*. Most of the feeding occurred on the branches of the top canopy of *P. pinaster* and *P. halepensis* and on the mid-canopy of *P. pinea* and *P. sylvestris*. All pine species were suitable for egg-laying, with *P. sylvestris* being the preferred. The divergence of results from these experiment under semi-natural conditions and from field observations is discussed. Overall, the four tested pines were found to be suitable hosts for the development of the insect and therefore can be inoculated with the PWN by the vector´s activity, suggesting the susceptibility of pine species to wilt disease is unrelated to the feeding and breeding selection of *M. galloprovincialis*.

Keywords: Pine wilt disease, *Bursaphelenchus xylophilus*, host suitability

PO557

ACQUISITION AND TRANSMISSION OF ´*CANDIDATUS PHYTOPLASMA MALI*’ BY APPLE PROLIFERATION INSECT VECTORS

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Apple proliferation (AP) is a phytoplasma-caused disease responsible for large economic damage in most European apple-growing areas. Two insect species, *Cacospylla picta* and *Cacopsylla melanoneura* (Hemiptera: Psyllidae), are known as vectors of ‘*Candidatus Phytoplasma mali*’, the etiological agent of AP. Studies conducted in different geographical regions suggested different transmission efficiencies for the two psyllids. Recently, a sudden AP outbreak was reported in apple orchards of northeastern Italy. The objective of the present study was to deepen the knowledge of the AP epidemiology in this area. Using field-collected overwintered adults, F1 nymphs and adults reared on infected apple plants and micro-propagated ‘Golden Delicious’ apple plants, we carried out acquisition and transmission experiments in two consecutive years under semi-field and greenhouse conditions, respectively. We found that the natural infection rates ranged between 1.1% and 2.7% in *C. melanoneura* and between 39.5% and 19.3% in *C. picta* in 2015 and 2016, respectively. F1 nymphs and adults of both AP vectors efficiently acquired the AP phytoplasma from infected plants. On average, ‘Ca. P. mali’ transmission was relatively low for both species in both years: *C. melanoneura* infected 2.6% and 5.1% of the apple plants, while *C. picta* reached an infection efficiency of 5% and 13.4%. Our findings are discussed in the light of future AP management strategies.

Keywords: Psyllid vectors, apple proliferation, phytoplasma, acquisition and transmission efficiency, ‘*Candidatus Phytoplasma mali*’

PO558

GENETIC DIVERSITY AND COMPETITION OF FLAVESCENCE DORÉE PHYTOPLASMA POPULATIONS BETWEEN INSECT VECTORS AND PLANTS

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Flavescence dorée phytoplasmas (FDp, 16SrV) are plant pathogenic non-cultivable bacteria associated with a severe and epidemic disease of grapevine and are transmitted by the Cicadellidae Deltocephalinae leafhoppers *Scaphoideus titanus* Ball (natural vector) and *Euscelidius variegatus* Kirschbaum (vector under laboratory conditions only). By living in two worlds, the plant and insect life style of phytoplasmas may drive differential gene expression, as known from literature, but also select different populations inside the plant and insect hosts. Genetic variation of phytoplasmas associated with FD (based on dnaK, vmpA and malG genes) in insects and plants revealed the presence of a wide range of genotypes infecting/co-infecting plants and vectors. FDp genetic variation is not homogeneous between insects and plants, as some genotypes are more represented in insects and others in plants. This skewed genotypic distribution indicates that insect vector drives phytoplasma populations that infect plants and, on the other way, host-plant drives the phytoplasma populations that can be acquired by and infect insects. The competition of the different genotypes inside plant and insect hosts and the presence of FD-infected plants inside and outside the vineyards, all of them representing source of inoculum for the vector, increase the complexity of this phytoplasma/plant/vector epidemiological system. Moreover, in the field, different plant species can harbour different FD phytoplasma genotypes and are visited by different vector/potential vector species. Some of these complex interrelationships have been studied at the field level as well as under controlled laboratory conditions. Although there is some convergence in the phytoplasma genotype composition infecting insects and plants at each site, a higher genetic variation of FD was consistently found in the insects compared to plants in all sites. Moreover, some genotypes were only found in vector or plant hosts. The ecological and evolutionary significance of this finding is discussed.

Keywords: Flavescence doréé phytoplasma, *Scaphoideus titanus*, grapevine, genotypes

Impact of Symbionts on Insect Biology

PO559

GENOME REDUCTION IN THE MOSQUITO SYMBIONT *ASAIA*

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The genus *Asaia* represents a peculiar member of Acetic acid bacteria (AAB): it oxidizes acetate and lactate to carbon dioxide and water, but not ethanol to acetic acid. Several species and strains of the genus *Asaia* were firstly isolated from tropical flowers. The symbiotic mutualism between *Asaia* and mosquitoes. encompassing Anopheles, Aedes and Culex genera, well-known vectors of parasites and arboviruses causing some of the major infectious diseases in public health, including malaria, Dengue and Zika is widely accepted and its colonization within its hosts is characterized by a wide tissue tropism, harbouring in the gut, the salivary glands and the reproductive tracts of female and male individuals, corresponding to the mode of its horizontal transmission (co-feeding) within a population and its vertical transmission between host generations (paternal transmission). Studies aimed at understanding the features of AAB symbiotic alliances with their hosts focused on the investigation of possible benefits provided by the role of the potential advantages given by AAB to their respective hosts. Through the genome sequencing of several *Asaia* isolates from various mosquito species, we disclosed differences in the architecture of the strain isolated from field-collected specimens of the South-American malaria vector *Anopheles darlingi*, and in particulary its genome reduction in comparison with other isolates from mosquito species belonging to Anopheles and Aedes genera. Thus *Asaia*, similarly to what proposed for *Serratia symbiotica* (seems to be a good model to study the genome reduction dynamics within a single bacterial taxon evolving in the mosquito host that represents a common biological niche.

Keywords: *Asaia*, endosymbiont, genome reduction

PO560

EFFECTS OF LAB PROCEDURES ON THE TRANSFER OF *B. OLEAE* ENDOSYMBIONT, *CANDIDATUS ERWINIA DACICOLA*

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Bactrocera oleae (Rossi) is a key pest of the olive crops in the Mediterranean basin. An important issue regarding *B. oleae* on which scientists are focusing the most is its symbiosis with *Candidatus Erwinia dacicola*. This bacterium is considered essential for the olive fly. It is vertically transmitted through generations and it benefits both larvae and adults in field; whereas, it had been found rarely in lab colonies, probably because of preservatives in artificial diets. So that, in order to understand the reason of its loss during rearing lab procedures, we exposed eggs laid by wild females to three different treatments: a propionic acid solution, a mixture of sodium hypochlorite plus Tritonxand water as control. The symbiont load on eggs was evaluated by real-time PCR and SEM. In the same time, we also tried a horizontal transfer among adults, exposing lab flies to ripe olives and gelled water contaminated by wild flies, wax domes containing eggs laid by wild females, cages dirtied by faeces dropped by wild flies in cohabitation with wild adults. In the first experiment, real-time PCR of eggs showed a significant reduction in Ca. *E. dacicola* abundance on eggs treated with propionic acid solution or the mixture of sodium hypochlorite plus Tritonxcompared to the control. Secondly, PCR-DGGE highlighted that the horizontal transfer of the endosymbiont occurred in the case of cohabitation. Thus, our results indicate how the symbiont load could be decreased by the use of preservatives in lab rearing procedures and that cohabitation between wild flies and lab ones permits the horizontal transfer opening further possibilities to establish a permanent symbiotic colony, a strategic tool for future SIT applications. Moreover, PCR-DGGE performed with an appropriate primer set was proved to be a consistent method for Ca. *E. dacicola* screening.

Keywords: Olive fly, lab rearing, endosymbiont, *Candidatus Erwinia dacicola*, DGGE, SIT

PO561

DIFFERENTIAL GENE EXPRESSION IN WOLBACHIA INFECTED AND NON-INFECTED *DROSOPHILA PAULISTORUM*

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The neotropical superspecies *Drosophila paulistorum* (Diptera, Insecta) comprises at least six reproductively isolated forms, each harboring a closely related but bidirectionally incompatible mutualistic Wolbachia strain. Although wild type flies of each semispecies are known to be isolated from the others by both pre- and post mating incompatibilities, recent studies have shown that mating and successful offspring development can be achieved after insects are treated for reduction of Wolbachia titer. This suggests that in this system the bacteria not only prevents embryonic development through cytoplasmic incompatibility but also affect mate choice, possibly through interference in pheromone production and recognition. In the present study, RNA samples were collected from heads and abdomens of males and females of three semispecies of *D. paulistorum*. Both naturally infected wild type specimens and flies which had gone through antibiotics treatment and subsequent gut flora restoration were sampled so that the influence of Wolbachia on gene expression could be investigated. Differential gene expression analysis was done using reads mapped to a de-novo assembled transcriptome and revealed hundreds of genes with altered expression after Wolbachia depletion. Several of these were involved in cell cycle and germ cell development, which possibly relates to Wolbachia's tropism for reproductive tissue and ability to maniputale host reproduction. However, a large number of genes related to other processes were also observed, including stress response, defense against microbes, pheromone production and reception, fatty acid metabolism, proteolysis, muscular and visual functions, among others which might be related to less studied phenotypes induced by the symbiont.

Keywords: Wolbachia, Drosophila, symbiosis

PO562

DIET AND DEVELOPMENTAL STAGE SHAPE THE BACTERIAL COMMUNITY DIVERSITY IN THE WASTE REDUCING INSECT *HERMETIA ILLUCENS* (L.)

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The microbiome associated to the insect gut is primarily involved in many aspects of the host biology and physiology, *e.g.* nutrition and immunity. While some host-intrinsic factors, such as the developmental stage and its genetics, are indirectly involved in shaping the microbiome structure and composition, the diet plays a direct effect on the assembly of the microbial partner community. Here, we evaluated the influence of three different diets (standard, fruit waste and vegetable waste) on the insect growth performance and on the structure and interactome of the bacterial microbiome associated to the black soldier fly (BSF), *Hermetia illucens* (Diptera: Stratiomyidae) along the host development (larvae, pupae and adults). BSF fed on organic wastes is indeed an interesting alternative source of proteins for livestock feed in the framework of the circular economy.
Diet significantly affected the host growth performances, as well as the physicochemical conditions (oxygen partial pressure, redox potential and pH) of BSF gut compartments. By combining the Automated Ribosomal Intergenic Spacer Analysis (ARISA)-PCR fingerprinting with the high-throughput sequencing of 16S rRNA gene amplicons, we analyzed BSF bacterial communities of individuals fed on different diets revealing the presence of a high bacterial diversity, with Proteobacteria, Firmicutes and Bacteroidetes as dominant phyla. Statistical analysis showed significant differences among the bacterial communities according to developmental stages and diets. Diet-specific bacterial components and keyplayers involved in the host sustenance were unveiled. Overall, the results indicated that both diet and developmental stage are driving forces in shaping BSF bacterial community and that the selection of diet-specific microbial clades could, ultimately, contribute to the host growth and development.

Keywords: Black soldier fly, bacterial community, 16S rRNA high-throughput sequencing

PO563

EVIDENCE OF FACULTATIVE ENDOSYMBIONT-MEDIATED PROTECTION AGAINST PARASITOIDS IN *BEMISIA TABACI* MED SPECIES AND PARASITOID COUNTERSTRATEGIES FOR COPING WITH HOST PROTECTION

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Inherited bacterial symbionts are common in arthropods and can have strong effects on the ecological interaction of their hosts. In addition to primary endosymbionts, members of the *B. tabaci* species complex have a diverse community of facultative endosymbionts, which can vary by species and geographical area. Facultative endosymbionts can affect *B. tabaci* fitness positively or negatively. For example, Rickettsia infecting *B. tabaci* MEAM1 species increases the host fitness and the tolerance to high temperatures, and favours a female biased host sex ratio. Facultative endosymbiont-induced protection against parasitoids has been showed in insects (*e.g.* aphids and Drosophila) and there are evidences of a Rickettsia-mediated protection in MEAM1. For invasive pests such as *B. tabaci* MED and MEAM1, the infections of facultative symbionts may accelerate the adaptation to new environments and intensify pest impact on agroecosystems. The endosymbiont Cardinium occurs in Mediterranean populations of the Q1 mitotype of *B. tabaci* MED, with infection frequency variable with geographic area. Analysis of the Cardinum genome suggested that this symbiont might function as a defensive symbiont against parasitoids. This prediction is supported by our laboratory results. We found evidence that Cardinium confers protection against the parasitoid *Eretmocerus mundus* in Q1 MED. We compared the *E. mundus* parasitism on two Q1 strains, with (Q1C+) and without (Q1C-) Cardinium infection, respectively and found that the parasitization level on Q1C- is significantly higher than on Q1C+. At the same time we found that *E. mundus*, when permanently reared on Cardinium-infected Q1 MED, showed high resistance to symbiont-induced host protection and became susceptible under relaxed selection pressure from Cardinium-infected hosts. This suggests a lower fitness of the *E. mundus* resistant genotype. Cardinium-mediated protection against parasitoids could favour the invasiveness of *B. tabaci* MED at least during the early phase of the colonization of new environments.

Keywords: *Bemisia tabaci*, Cardinium, facultative endosymbionts, *Eretmocerus mundus*, protective symbionts, whiteflies

PO564

SPATIO-TEMPORAL CHANGES IN THE GUT MICROBIOTA OF *POPILLIA JAPONICA*

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The number of invasive insect species is increasing and, once established, they have serious impacts on the environment and economy. *Popillia japonica*, also known as Japanese beetle, is a polyphagous pest with larvae consuming roots while adults the plant leaves. Native of Japan, the beetle was able to spread to different countries, including Italy, and was thus classified by EPPO as A2 pest. Insect-associated bacteria have shown to play important roles for the host evolutionary success and adaptation. In this study, we investigate the microbiota associated with the gut regions of *P. japonica* developmental stages. In order to carry out the study, 90 individuals were dissected and pooled into 18 samples representing different developmental stages and gut regions of the insect. We amplified and sequenced using Illumina platform the bacterial 16S rRNA gene; the reads were analyzed using QIIME. Our results showed that *P. japonica* harbors a complex and diverse gut microbiota (average 262 OTUs), with Bacteroides dominating the communities of adults. Within the developmental stages, the larvae resulted to harbor a more diverse bacterial community (average 289 OTUs), while the most diverse gut region was the hindgut (average 258 OTUs). From an initial analysis, no core microbiota seems to be shared among the developmental stages. Nonetheless, within a specific stage it is possible to identify a specific entomotype (*e.g.*, Clostridiales were abundantly present in all larval hindguts). The cluster analysis showed that the microbiotas associated with larvae clustered together, well separated from adult's gut communities; on the other hand, pupae gut bacterial communities were spread in the continuum from larvae to adults. These results

suggest that the developmental stage (*i.e.*, larvae *vs* adults) might have an influence in shaping the microbiota of *P. japonica*, although this is not visible when different larval instars are compared.

Keywords: Insect symbiosis, invasive species, microbiota, 16S rRNA profiling

PO565

RAMPANT HOST-SWITCHING SHAPED THE TERMITE GUT MICROBIOME

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The gut microbiota of animals exert major effects on host biology. Although horizontal transfer is generally considered the prevalent route for the acquisition of gut bacteria in mammals, some bacterial lineages have co-speciated with their hosts on timescales of several million years. Termites harbor a complex gut microbiota, and their advanced social behavior provides the potential for long-term vertical symbiont transmission, and co-evolution of gut symbionts and host. Despite clear evolutionary patterns in the gut microbiota of termites, a consensus on how microbial communities were assembled during termite diversification has yet to be reached. Although some studies have concluded that vertical transmission has played a major role, others indicate that diet and gut microenvironment have been the primary determinants shaping microbial communities in termite guts. To address this issue, we examined the gut microbiota of 94 termite species, through 16S rRNA metabarcoding. We analyzed the phylogeny of 211 bacterial lineages obtained from termite guts, including their closest relatives from other environments, which were identified using BLAST. The results provided strong evidence for rampant horizontal transfer of gut bacteria between termite host lineages. While the majority of termite-derived phylotypes formed large monophyletic groups, indicating high levels of niche specialization, numerous other clades were interspersed with bacterial lineages from the guts of other animals. Our results indicate that ‘mixed-mode’ transmission, which combines colony-to-offspring vertical transmission with horizontal colony-to-colony transfer, has been the primary driving force shaping the gut microbiota of termites.

Keywords: Symbiosis, eusociality, horizontal symbiont transfer

PO566

MOLECULAR CHARACTERIZATION OF APHID BACTERIOCYTE CELL DEATH BY RNASEQ ANALYSIS

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Beneficial symbiotic associations, ubiquitously found in nature, have led to the emergence of novel eukaryotic cells, the bacteriocytes, specialized in harboring microbial partners. One of the most fundamental questions concerning these enigmatic cells is how organismal homeostasis controls their elimination in response to host physiology. We recently discovered that in the hemipteran insect *Acyrthosiphon pisum* bacteriocytes degenerate through a multistep cell-death process activated progressively during aphid adulthood and presenting morphological features distinct from evolutionarily conserved cell-death mechanisms, such as autophagic or apoptotic cell deaths. To decipher the molecular mechanisms regulating this hitherto unknown cell death process, we performed RNA-seq analysis of *A. pisum* bacteriocytes at key stages of the insect life cycle, using bacteriocytes isolated from 9-, 15- and 23-day-old aphids. We were able to show that induction of endoplasmic reticulum stress genes is concomitant with the early cytoplasmic hypervacuolation that marks the beginning of aphid bacteriocyte cell death. This is followed by the activation of general cell stress responses, including the induction of Autophagy related (ATG) genes. At late stages of bacteriocyte degeneration, we also observed an increased expression of pro-apoptotic genes and a simultaneous significantly increased expression of apoptosis inhibitor genes. The last step of aphid bacteriocyte cell death is a potent activation of the lysosomal system, with the induction of most of the *A. pisum* lysosomal genes and of lysosome-associated Rabs. These results confirm that aphid bacteriocyte cell death is autophagy and apoptosis-independent as these pathways are activated after the initial event of bacteriocyte cell death, *i.e.* the cytoplasmic hypervacuolation. They also furnish a possible mechanistic explanation for the absence of morphological signatures for apoptosis in degenerative bacteriocytes, namely by potentially being blocked by upregulated apoptosis inhibitors. Our findings provide a framework to study biological functioning of bacteriocytes and the molecular and cellular mechanisms associated with symbiosis.

Keywords: Symbiosis, aphids, bacteriocyte cell death, RNAseq

PO567

EUSCELIDIUS VARIEGATUS IFLAVIRUS 1: INSECT-TO-INSECT TRANSMISSION

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Euscelidius variegatus Kirschbaum (Hemiptera Cicadellidae) is a well-known leafhopper vector of phytoplasmas. A new member of a new species of the genus Iflavirus was recently discovered during transcriptomic analyses of a laboratory population of *E. variegatus*, and named *E. variegatus* virus 1 (EVV1). EVV1 infection was asymptomatic, and the virus was constantly detected both in phytoplasma exposed and not exposed vectors. Interestingly, virus load was significantly lower in the former category. Two other *E. variegatus* populations from France and US were found to be virus free (EVV1-), and one virus free population was therefore established in our lab starting from the French one. As Iflaviruses are generally known to infect the midgut of insects, preliminary *in situ* hybridization experiments confirmed EVV1 localization in the gut of *E. variegatus*, and this observation suggested that the virus might be secreted in the honeydew by infected leafhoppers. A protocol was then adapted from the available literature and the results confirmed the presence of EVV1 in the honeydew of infected insects. Horizontal transmission of the virus to the virus-free population was then tested through either artificial feeding on honeydew-contaminated feeding medium or through feeding on virus-contaminated plants from EVV1+ rearings. EVV1 was also found in the eggs of the virus-infected population, suggesting vertical transmission to the progeny. Individuals of the EVV1- population were positive for virus presence, following abdominal microinjection with crude sap obtained by crushing of EVV1+ insects. Phytoplasma vector ability of EVV1+ and – populations with the same genetic background will be carried out to provide clues on the possible three-way interactions among insect/virus/phytoplasma. The production of an infectious EVV1 clone to express heterologous genes in the leafhopper and manipulate the expression of endogenous genes by virus-induced gene silencing is ongoing.

Keywords: EVV1, leafhopper, vector, phytoplasma

PO568

COMMENSAL MICROBIOTA MODULATES LARVAL FORAGING BEHAVIOUR AND DEVELOPMENTAL TRAITS IN *BACTROCERA TRYONI*

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Commensal microbes help the juvenile insects to survive and growth during development, and can have long-lasting fitness implications to individuals in adulthood. Larvae acquire commensal microbes from the previous generation (*i.e.* vertical transmission through the egg) and from the larval environment (*e.g.* food source). To date, however, how vertical transmission and microbe acquisition from the diet interact to shape insect development remains largely unknown. In this study, we investigated how vertically transmitted microbes and microbes acquired by the larvae influenced key developmental factors in the major Australian horticulture pest, the Queensland fruit fly *Bactrocera tryoni*. Our results revealed that both microbes from the egg and microbes acquired by the larvae are needed for high egg hatching and larval pupation. Microbes acquired during larval stage modulated larval foraging behaviour to intermediate and sugar-biased diets, whereas the presence of vertically transmitted and acquired microbes modulated larval foraging behaviour to intermediate and protein-biased diets. Importantly, both microbes in the egg and acquired by the larvae are necessary to maximise larval growth, and only microbes acquired by the larvae cannot fully rescue larval growth. Furthermore, larval growth was differentially affected by the microbe load, in which larval body mass increased when the microbial load was intermediate but decreased when the microbial load was high. Together, our findings highlight the far-reaching effects of the microbiota on early development of an important agricultural pest species.

Keywords: Nutrition, Larval behaviour, Development, Microbial Ecology, Agricultural pest

PO569

SYMBIONT-MEDIATED CHEMICAL DEFENSE IN LADYBIRDS?

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All organisms use chemical agents to communicate with their environment. Many of these highly efficient so called semiochemicals belong to a large group of volatile alkylpyrazines, particularly methyl- and methoxypyrazines (MPs). MPs are generated by a wide range of aposematic and/or toxic insects. Besides their widespread function as alerting signals to pretend predators, MPs are applied particularly in ladybirds also as pheromones supporting intraspecific communication, attraction and even aggregation behavior. However, up to now little is known about the production site of MPs in insects. Do they sequester MPs from their hosts (plants) or prey or do they produce them (or their precursors) by themselves in specific organs or tissues? To elucidate the derivation of the MPs we studied the harlequin lady beetle *Harmonia axyridis*, which generates different MPs. Given that pyrazines are the most common semiochemicals produced by bacteria and that the MP-concentration in adult *H. axyridis* depends on the larval feeding behavior, it is possible that the gut microflora may participate in the generation of MPs. Our headspace analysis showed that not the presumed fat body tissue, but the gut of *H. axyridis* may take a role in the generation of MPs, because it revealed the second-highest MP-content after the hemolymph as the presumed storage site. Furthermore, the application of antibiotics to the diet results in a reduced MP-content. Sequence analysis of the gut microbiota revealed several bacteria which are reportedly able to produce MPs. Our experiments indicate a potential symbiotic relationship between the host and its gut microbiota that generates MP volatiles which considerably influence ladybird behavior and survival.

Keywords: Chemical defense, methoxypyrazines (MPs), *Harmonia axyridis*, gut microbiota

PO570

POTENTIAL INFLUENCE OF THE MICROBIAL COMMUNITY OF *CACOPSYLLA MELANONEURA* (HEMIPTERA: PSYLLIDAE) IN PHYTOPLASMA TRANSMISSION

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Phytoplasmas are bacterial pathogens that are responsible for a wide range of diseases of various plant species. In nature, phytoplasmas are primarily transmitted by phloem sucking insects. Apple proliferation (AP) is a disease of apple trees in Europe caused by the phytoplasma 'Candidatus P. mali'. AP phytoplasma is causing proliferation of auxiliary shoots ("witches' broom"), decreased in fruit size and quality and is responsible for vast economic damages especially in the apple growing regions of Northern Italy. *Cacopsylla melanoneura* (Hemiptera: Psyllidae) is a vector of AP phytoplasma with varying transmission efficiencies. Knowledge of the factors that are influencing phytoplasma transmission is currently limited. The microbial community of the insect vectors is an important factor that might influence the acquisition and transmission rate of phytoplasma. Here we present data on the high-throughput metagenomic analysis of the 16S rRNA of field collected *C. melanoneura* individuals. We show differences in the microbial community of 'Ca. P. mali' infected and uninfected individuals of *C. melanoneura*. We discuss how these microbes might influence the transmission of phytoplasma and how microbes can be used as potential biological control agent of AP.

Keywords: Psyllid, Insect vector, Apple proliferation, Phytoplasma, Endosymbiont

PO571

A NOVEL CELL DEATH PROCESS ELIMINATES BOTH BACTERIOCYTES AND THEIR SYMBIONTS IN THE PEA APHID/BUCHNERA SYMBIOTIC SYSTEM

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Symbiosis is a key source of ecological and evolutionary diversification of eukaryotic organisms throughout the animal and plant kingdoms. In insects that are obligatorily dependent on intracellular bacterial symbionts, novel host cells, the bacteriocytes, have evolved for harboring beneficial microbial partners. These cells constitute a fascinating riddle in developmental cell biology, as their embryonic origin and the molecular mechanisms governing their development and organogenesis, as well as their elimination in response to host physiology, remain largely unsolved. Here we report the discovery of a hitherto unknown cell-death process involved in the degeneration of bacteriocytes in the pea aphid *Acyrthosiphon pisum*/*Buchnera aphidicola* symbiotic system, which emerged in recent years as a powerful model for studying symbiotic relationships. This new form of cell death is activated progressively throughout aphid adulthood and exhibits morphological features distinct from evolutionary conserved pathways, including apoptosis- or autophagy-dependent cell deaths. By combining electron microscopy, immunohistochemistry, and molecular analyses, we demonstrated that the initial event of bacteriocyte cell death is the cytoplasmic accumulation of non-autophagic vacuoles, followed by a sequence of cellular stress responses including the formation of autophagosomes in intervacuolar spaces, swelling of bacteriocyte mitochondria, activation of reactive oxygen species, and *Buchnera aphidicola* endosymbiont degradation by the lysosomal system. This multistep cell-death process originates from the endoplasmic reticulum, an organelle exhibiting a unique reticular network organization in these cells, probably imposed by the presence of millions of Buchnera endosymbionts in each bacteriocyte (Simonet *et al.*, 2018, PNAS, doi: 10.1073/pnas.1720237115). Our findings reveal novel mechanisms by which both bacteriocyte cell and symbiotic bacterial numbers are controlled to maintain organismal homeostasis. They also shed light on previously unknown consequences of persistent obligatory symbiotic bacteria on the cellular organization and functioning of bacteriocytes in their eukaryotic hosts.

Keywords: Symbiosis, *Acyrthosiphon pisum*, *Buchnera aphidicola*, bacteriocytes, cell death

PO572

HOST-MICROBE INTERACTIONS BETWEEN THE TSETSE INNATE IMMUNE SYSTEM AND THE SECONDARY SYMBIONT *SODALIS GLOSSINIDIUS*

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Tsetse flies are the sole vectors of protozoan parasites of the genus *Trypanosoma*, which cause human and animal African trypanosomiasis. Additionally, tsetse harbors a low diversity microbiome with varying degrees of integration, making them an ideal model to study symbiont-host interactions. The tsetse's secondary symbiont *Sodalis glossinidius* is in an intermediate state towards symbiosis and capable to be cultivated. We used this symbiont as a model to understand molecular mechanisms that govern endosymbiont-host immune interactions. Specific elimination of Sodalis in tsetse followed by re-infection allowed investigating how the secondary symbiont is perceived by its host. Through RNA-sequencing (RNA-seq) whole transcriptomics, we found that Sodalis triggered moderate host responses, affecting only 34 tsetse genes. On the other hand, tsetse infection with *E. coli* resulted in 1,495 altered genes. This clearly demonstrated the capacity of the flies to distinguish between Sodalis and the exogenous *E. coli*. In-depth analysis of host immunity genes indicated a benign immune response after re-infection with Sodalis, reflected by antimicrobial peptides (AMPs) expression many times lower compared to *E. coli*. This supports the hypothesis of a fine-tuned tsetse response to promote a stable association with Sodalis, while combatting the exogenous *E. coli*. Functional experiments demonstrated that Sodalis remains unaffected after RNA-interference (RNAi)-mediated suppression of the host immune deficiency (IMD)-pathway. Moreover, symbiont densities are unaffected *in vivo*, after host immune activation. These results indicated that humoral immune pathways do not play a major role in regulating Sodalis titers and that Sodalis is not effectively recognized by its tsetse host. Our study provides first insights in the interaction between Sodalis and the tsetse immune system. At a broad level, this fundamental knowledge generated on the tsetse-Sodalis interaction could serve as a paradigm for other endosymbiont-insect interactions that are not cultivable and less amenable to genetic studies.

Keywords: Secondary symbiont, innate immunity, *tsetse fly*

PO573

NOTES ON CLADOSPORIUM SPECIES ASSOCIATED WITH ASPHONDYLIA GALLS ON SPECIES OF LAMIACEAE IN POLAND AND IN ITALY

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Recent joint activities carried out by our research groups concerning Asphondylia gall midges and associated fungi on several species belonging to the Lamiaceae confirmed the constant occurrence of *Botryosphaeria dothidea* as the fungal symbiont of midges both in Poland and in Italy. However, isolations from galls also yielded *Cladosporium* spp. in high frequencies, with an uncertain symbiotic role. In the past, these fungi were reported as the main inquilines in Asphondylia galls on plants belonging to different botanical families; these combined remarks stimulate a more accurate

consideration of Cladosporium strains in order to assess if they eventually play a role in this complex insect-fungus association. Morphological observations indicated that the majority of our isolates belong in the *C. cladosporioides* species complex, which is reported to include many closely related taxa and a good number of cryptic species yet to be described. Considering our opportunity to collect strains from quite a peculiar biocenotic context, as an additional contribution to the taxonomic resolution of this species complex we further characterized a sample of 47 strains through a phylogenetic analysis based on DNA sequences from the rDNA-ITS region (ITS1, 5.8 rDNA gene, ITS2), and loci coding for actin (Act) and the translation elongation factor 1-α (TEF). Particularly, Act sequence homology was effective in settling this assortment, leading to the ascription of a number of isolates to infrequent species, such as *C. allicinum*, *C. basiinflatum* and *C. ramotenellum*, and to the identification of four well defined isolate clusters possibly representing novel species.

Keywords: Asphondylia gall midges, Cladosporium, Lamiaceae, symbiosis”