Population dynamics of Fusarium graminearum in various crop residues using qPCR measurements

Johann Leplat, Muhammad Abid, Cécile Héraud, Leon Fayolle, Elodie Gautheron, Véronique Edel-Hermann, Pierre P. Mangin, Laurent Falchetto, Christian Steinberg

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Abstract: *Fusarium graminearum* causes wheat head blight disease and produce different mycotoxins (as Deoxynivalenol=DON) which are toxic for human and animal. During off season it survives in the soil, on weeds and in crop residues. A 6 months study was conducted in controlled conditions (microcosms of natural soil, 17°C, 80%WHC) to test whether the presence of DON in the wheat crop residues gives advantages to *F. graminearum* to survive and develop a primary inoculum during the decomposition process. This study was carried out in the presence of the whole soil biota (i.e. fungi, bacteria, protozoa, nematodes and earthworms). Both *F. graminearum* and the biota were monitored during the decomposition process. The wheat crop residues were placed on the soil surface or incorporated into the natural soil. This experiment was conducted with (1mg DON/kg soil-straw mixture) and without DON. This study suggested that the mycotoxins provided a competitive advantage to *F. graminearum* and were deleterious towards the fungal community and the nematodes but not towards the bacteria or the earthworms. However, all the results were not statistically significant. The use of molecular tools as well as the forthcoming quantification of DON in the residues during 24 weeks of observation will provide the clarification and may confirm or not the trends we observed.

Abstract: Oilseed rape (OSR) grown frequently in rotation suffers from yield decline. Molecular techniques have been used to identify changes in the rhizosphere fungal communities of OSR grown in different rotational frequencies with wheat. In particular, *Olpidium brassicae* and *Pyrenochaeta* sp. were found to increase in relative abundance in rhizosphere fungal communities of OSR cropped continuously or in short rotation, compared to OSR grown in long rotations. In order to investigate whether these two microorganisms contribute to yield decline, working cultures were isolated from field samples and controlled glasshouse experiments were carried out. For this glasshouse work, a model plant species (*Brassica oleracea;* closely related to OSR) was used to facilitate rapid seed production under glasshouse conditions. Experiments were initially carried out in a soil-less medium (sand-terragreen mix) to assess the impact of the individual fungal species alone on the plants. Plants were inoculated with a range of doses of the two fungi in separate experiments, and results from this model system indicated that both species affected plant growth in terms of either reduced branching (*O. brassicae*) or reduced seed quality and quantity per pod (*Pyrenochaeta* sp.).

From metagenome and proteome to biocontrol: analysis of moss- and lichen-associated microbial communities
Gabriele Berg, Anastasia Bragina, Massimiliano Cardinale, Christin Zachow, Henry Müller, Christian Berg, Andrej Shcherbakov, Wladimir Chebotar, Martin Grube 13-16

Abstract: Bryophytes and lichens represent interesting bioresources; their tight interaction with microorganisms is only partly understood. Recently it was shown that both groups of cryptogams are colonized at high abundance with special microbial communities, which functionally contribute to the vitality of moss plantlets and lichen symbioses. To characterize biodiversity of associated microbial communities, we applied combination of methods: phylogenetic diversity of clone libraries and metagenomic and proteomic approaches. In addition, isolates were investigated regarding their interaction with plants. According to our results, cryptogam-associated microbial communities comprise a unique pool of highly diverse, mostly uncultivable and host-specific microorganisms which community structure strongly depends on abiotic and biotic factors. Additionally, bacteria were isolated for biotechnological applications. A high proportion of isolates showed antifungal activities and a remarkable plant growth promotion by nitrogen fixation, phosphate solubilisation and production of phytohormons as well as high biocontrol potential.

Global ecological patterns in uncultured Archaea: Do they have a role in multitrophic interactions in soil?
Emilio O. Casamayor 17

Abstract only

Will climate change influence multitrophic interactions in soil?
Sukumar Chakraborty 18

Abstract only

Effect of weeds on microbial community in vineyards soil
Paola Elisa Corneo, Alberto Pellegrini, Cesare Gessler, Ilaria Pertot 19-22

Abstract: Weeds, in particular agrestals, represent a threat for a variety of cultivated plants, because they compete for nutrients, water and sunlight. In addition they may affect the crops by producing toxic compounds through a mechanism called allelopathy. Their presence leads to huge economical losses, but on the other hand their control, especially through herbicides, could negatively affect the environment. Therefore weed control through different strategies of prevention, control and eradication by means of sustainable approaches is a priority worldwide. Almost nothing is known on the interaction between weeds and soil microorganisms, for example if weeds could play a role in the interaction with beneficial soil microbes and in preserving soil microbiological quality. In this study we determine the effect of different weeds on total bacterial and fungal abundance in different soils under controlled conditions. We collected soil samples in four vineyards in northern Italy and three weeds, *Poa trivialis, Taraxacum officinale* and *Trifolium repens*, were selected based on their ubiquitous presence in the original soils. Each weed was planted in each soil type. The total amount of fungi and bacteria during different plant stages development was assessed. Total fungi are poorly affected by the plant introduction, even if, at the true leaf stage of *T. officinale* and *T. repens,* an increase was observed. Total bacteria community at true leaf stage also showed a significant increment with *T. officinale* and *T. repens* in some soils. The monocotyledon *P. trivialis* globally did not affect the bacterial and fungal communities. Even if a general trend cannot be inferred, we demonstrate an interaction with the combination of weed species and soil.

Biochar soil amendement: pinpointing microbial elucidators of induced systemic plant resistance
Max Kolton, Yigal Elad, Ellen Graber, Yael Meller-Harel, Zohar Pasternak, Eddie Cyrtryn 23-26

Abstract: Application of biochar to soil results in long term sequestration of fixed carbon as well as improved soil quality and crop productivity. Furthermore, recent studies conducted in our laboratory indicate that soil-applied biochar promotes systemic resistance of plants to several prominent foliar pathogens in a variety of crops such as tomato, pepper and strawberry. We hypothesize that this phenomenon may be at least partially attributed to root-associated microbial elicitors whose presence is somehow augmented in the biochar-amended soils. To explore this hypothesis, we compared the bacterial community composition on roots of 3-month old pepper plants in biochar-amended and non-amended potting mixtures, using pyrosequencing of 16S rRNA gene tags. *Flavobacterium* was the most abundant genus detected on the roots, and relative abundance of this group was almost three-fold higher in the biochar-amended samples then in the non-amended samples. Research is currently focusing on the direct biocontrol capacity of root-associated flavobacterial isolates toward phytopathogenic fungi and nematodes and their potential to induce resistance in plants against foliar pathogens.

Multi-pathogen disease caused by *Didymella bryoniae* and bacteria on Styrian oil pumpkin: microbial ecology and biocontrol
Michael Fürnkranz, Eveline Adam, Birgit Lukesch, Henry Müller, Sabine Zitzenbacher, Martin Grube, Gabriele Berg 27-30

Abstract: The Styrian oil pumpkin, *Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Greb., is a crop of cultural, medical and commercial importance in Austria. Since 2004, fruit rot and black rot caused by the ascomycete *Didymella bryoniae* (Fuckel) Rehm has lead to dramatic yield losses. In the field, the fungal disease was usually associated with characteristic symptoms of bacteriosis. Bacterial pathogens included *Pectobacterium carotovorum, Pseudomonas viridiflava, Pseudomonas syringae* and *Xanthomonas cucurbitae.* The high coincidence of fungal and bacterial disease suggests mutualistic effects in pathogenesis. By cultivation-dependent methods, the fraction of Styrian oil pumpkin inhabiting *in vitro* antagonists against *D. bryoniae* and bacterial pathogens was investigated and ended in an ARDRA and BOX PCR based selection of five bacterial broad-spectrum antagonists from 2.320 initially tested microbes: strains of *Lysobacter* spp., *Pseudomonas chlororaphis, Paenibacillus polymyxa* and *Serratia plymuthica* were the favorites. They were successfully evaluated in greenhouse and field trials. On their basis, a biological product to protect the Styrian oil pumpkin against microbial diseases will be developed.

Protection against a vine trunk attack by *Phaeomoniella chlamydospora* is concomitant with root colonization by the oomycete *Pythium oligandrum*
Jonathan Gerbore, Emilie Bruez, Jessica Vallance, Damien Grizard, Catherine Regnault-Roger, Patrice Rey 31-35

Abstract: Strains of the biocontrol agent, *Pythium oligandrum*, have been isolated from various vineyards of the Bordeaux region and ability of the oomycete to protect vines from attacks by *Phaeomoniella chlamydospora* (Pch) has been assessed. Pch is a fungus associated with Esca decline of vines and it induces wood lesions in the trunk of plants. In our experiment roots were sampled from vine plants grown in three different soils, *i.e.* silt-clay, sandy-clay, stony. Whatever the conditions, *P. oligandrum* strains were isolated from 11 out of 12 samples. It seems therefore well ecologically adapted to vineyard soils. Forty strains were isolated and purified. *P. oligandrum* strains were identified by sequencing of the rDNA repeat unit, *i.e.* 3'end of the 18s rRNA gene, ITS-1, 5.8s rRNA gene, ITS-2, and around 580 bp of the 5'end of the 28S rRNA. *P. oligandrum* elicitor detections have been carried out by amplification of two genes: oligandrin and POD1. They were detected in most of the strains. Subsequently, some *P. oligandrum* strains were used to carry out experiment about vine protection. Cabernet Sauvignon vine cuttings were first inoculated at the root level with *P. oligandrum* strains, then one week later trunk of young plants were inoculated with Pch. The Pch strain used was chosen because of its aggressiveness and its ability to induce necrosis in wood. Protection of up to 50% was obtained when the rhizosphere of vine cuttings was colonized with *P. oligandrum*. These observations provide the first convincing evidence that *P. oligandrum* has the potential to induce plant resistance in wood of vine and that it should be useful for the biological control of grapevine wood disease.

Abstract only

Abstract: Dagger and needle nematodes are considered globally one of the 10 most economically important nematode groups. They interact with the plants by direct damage in feeding process from them or in plant virus transmission. The accurate and timely identification of dagger and needle nematodes infecting vineyards is a prerequisite for designing effective management strategies. This is particularly relevant in grapevine fields because of the diversity of these nematodes and the need for distinction between virus vector and non-virus vector nematodes. This present study establishes the biodiversity of longidorids found in grapevines at southern Spain, as well as their integrative diagnosis and molecular phylogenetic relationships, using morphometrical analysis and molecular markers including D2-D3 expansion regions of 28S rRNA, 18S rRNA and ITS1-rRNA. *Xiphinema pachtaicum* and *X. index* were the most frequently dagger nematodes found (90.8 and 30.3% frequency of infestation, respectively), but other dagger species included *X. adenohystherum*, *X. hispidum*, *X. italiae*, *X. lupini*, *X. nuragicum*, *X. rivesi*, and *X. turcicum* (1.3%, 7.9%, 13.2%, 3.9%, 1.3%, 2.6%, and 1.3% frequency of infestation, respectively). Needle nematodes were less abundant and diverse including only *Longidorus alvegus* and *L. magnus* (2.6% and 11.8% frequency of infestation, respectively). The species identification based on sequencing of rRNA regions and BLAST analysis was congruent with species identification based on morphometrical studies. D2-D3 expansion segments of 28S rDNA and ITS1 were most useful for dagger and needle nematode species identification since they showed more variability than partial 18S rDNA. Results also showed a high level of nematode endemism (such as *X. adenohystherum*, *X. hispidum*, *X. nuragicum*, *X. lupini* and *L. alvegus*) and their phylogenetic grouping suggest a common origin for several of the longidorid species found and the Iberian Peninsula as their potential centre of origin. Phylogeny showed two major clades well defined and supported including *Longidorus* and *Xiphinema* species. However, the grouping of *Longidorus* and *Paralongidorus* species with the rest of species is well supported. Nevertheless, tree topology analysis by Shimodaira-Hasegawa test of D2-D3 and partial 18S of our broad sequences did not refute the monophyly of the genus *Xiphinema*, but the genus *Paralongidorus* was not accepted as a valid.

Abstract: In recent years alterations of chemical signals from plants and/or microbes have received increasing attention, as they are considered to play a central role in determining the positive or negative outcome of interactions in the rhizosphere. However, there is still a lack in the understanding how root exudates affect the development of fungal propagules in the rhizosphere. In this work a complex system consisting of tomato (*Solanum lycopersicum* L.), the intercropping partner leek (*Allium porrum* L.), the soilborne tomato pathogen *Fusarium oxysporum* f.sp. *lycopersici* (FOL) and the arbuscular mycorrhizal fungus *Glomus mosseae* (AMF) was designed to assess metabolic changes in root exudation and the effects of these exudates on FOL. Tomato and leek plants were grown together in pots and inoculated with FOL, AMF, FOL and AMF in combination. Uninoculated tomato and leek plants and two tomato plants grown together were added as control treatments. After a period of 10 weeks under greenhouse conditions root exudates were collected. The exudates were used for in vitro tests with FOL (e.g. spore germination, activity) and for analysing the components (e.g. sugars, secondary metabolites) of the exudates with HPLC-DAD and GC-MS, respectively. Furthermore, data about root weights, AMF colonization rates and FOL infection rates will be discussed.

Induced systemic resistance in strawberry (*Fragaria × ananassa*) to powdery mildew using various control agents

Yael Meller Harel, Max Kolton, Yigal Elad, Dalia Rav-David, Eddie Cytryn, David Ezra, Menahem Borenstein, Ran Shulchani, Ellen R. Graber 47-51

Abstract: Powdery mildew, caused by *Podosphaera aphanis*, is one of the major fungal diseases of cultivated strawberry (*Fragaria* × *ananassa* Duchense) world-wide**.** In the present research we report the efficacy of various control agents in induced resistance in strawberry plants against Powdery mildew. Inducers tested are *Trichoderma harzianum* T39, acibenzolar-S-methyl (Bion), a new protein hydrolysate inducer, an isolate of a bacterium and one of a yeast, and biochar. These agents induced systemic resistance when applied to the root zone, while the disease was found and measured on the leaves. In addition, we studied the effect of T39, Bion and biochar on plant defense related gene expression. We show that while Bion induced Systemic Acquired Resistance (SAR) only, as expected, T39 as well as biochar induced SAR and Induced Systemic Resistance (ISR).

Respiratory path between plant and soil

Hartmut Heilmann 53-57

Abstract: Electrolytes usually are not looked upon as relevant for soils and for plant growth. As well electrochemical activity in the soil is not taken into consideration. This is also true for the inner respiration and energy flow in the organic unit of a soil site which can be looked upon as an organism. Work on these fields conveyed that there is a respiratory path between plant and soil in a changing polarity between day and night. This phenomenon makes the electrochemical fertilization effect plausible. System modelling shows that plants get energy from anion respiration from the soil and the soil site in its life cycle can regain energy both from sun and soil. The energy from soil is conveyed by the pressure of highly active oxidative pressure of anion activity. This not only leads to higher yields but also to higher insect calamities.

Detection and diversity of nematodes in the rhizosphere and bulk soil of oilseed rape and wheat grown in shortened rotations

Sally Hilton, Amanda J. Bennett, Gary Bending, David Chandler, Peter Mills 59-63

Abstract: A long-term field experiment of OSR grown in a range of rotations with wheat was used to determine whether shifts in nematode populations of the rhizosphere and bulk soil could be identified. DNA was extracted from the rhizosphere and the bulk soil, and nematode communities were profiled by Terminal Restriction Fragment Length Polymorphism (TRFLP) using nematode specific primers to the 18s ribosomal RNA gene. TRFs were identified by construction of a clone library. Nine different families of nematodes were detected in the clone library from the continuously cropped OSR rhizosphere, including three families containing known plant pathogens, including *Pratylenchus neglectus* (root lesion nematode), *Coslenchus franklinae, Bitylenchus dubius* and *Tylenchorhynchus dubius.* There was a significant difference between the nematode communities in the rhizosphere of the different crops, but there was no difference in the bulk soil. There was also a significant difference between the nematode communities of the rhizosphere soil and bulk soil, due mainly to an increase in the relative abundance of plant pathogenic nematodes in the rhizosphere, in particular *Pratylenchus neglectus*. Further primers were designed to amplify the cyst nematodes within the genera *Heterodera*. The TRF corresponding to these pathogenic nematodes was not detected in this field trial, but has been detected at high levels in another site within the UK.

Verticillium wilt of olives in southern Spain: A case study for the impact of changes in cropping practices on development and management of soil-borne plant pathogens
Rafael M. Jiménez-Díaz 65-66
Abstract only

Population dynamics of *Fusarium graminearum* in various crop residues using qPCR measurements
Johann Leplat, Muhammad Abid, Cécile Heraud, Léon Fayolle,
Elodie Gautheron, Véronique Edel-Hermann, Pierre Mangin,
Laurent Falchetto, Christian Steinberg 67-71
Abstract: The influence of biotic (disinfected and non disinfected soil) and trophic (presence of crop residues or not) factors as well as the nature of residues (wheat, maize, rape) on the saprotrophic competence of *Fusarium graminearum* in soil was investigated in controlled conditions in soil microcosms for 3 months using qPCR to monitor the population dynamics of the plant pathogenic fungus. The main factor was soil disinfection establishing that the survival of *F. graminearum* was regulated by soil microbial communities. Crop residues provided a favourable habitat for *F. graminearum* but the growth of the pathogenic fungus was promoted to different extents according to the origin of the residues.

Involvement of Induced Systemic Resistance in control of Verticillium wilt by fluorescent *Pseudomonas* spp.
Mercedes Maldonado-González, Peter A. H. M. Bakker, Jesús Mercado-Blanco ... 73-76
Abstract: Verticillium wilt, caused by *Verticillium dahliae* Kleb., is a problem in many crops and the disease is difficult to control. Strains of *Pseudomonas fluorescens* and *P. putida* were previously isolated from root tissues of olive trees, cv. Picual. Some of them are endophytic and can control the highly-virulent, defoliating (D) pathotype of *V. dahliae* in olive. One mode of action of disease suppression by fluorescent *Pseudomonas* spp. is induced systemic resistance (ISR). *Pseudomonas* spp. strains were tested for ISR in a system using *Arabidopsis thaliana* and the pathogens *P. syringae* pv. *tomato* and *Botrytis cinerea*. To include *V. dahliae* in these studies we inoculated *A. thaliana* Col-0 with several isolates of this pathogen belonging to different vegetative compatibility groups (VCG). Isolate V937I (VCG1A, D pathotype) produced severe symptoms in Arabidopsis, and *P. fluorescens* PICF7 was able to control the disease caused by this virulent isolate. The use of non-ISR expressing accessions and mutants of *A. thaliana* will allow to evaluate involvement of ISR in control of Verticillium wilt.

Biological and physiological aspects in the host-parasite relationship of the nematophagous fungus *Pochonia chlamydosporia*
Rosa H. Manzanilla-López, Ivania Esteves, Stephen J. Powers, Brian R. Kerry 77-80
Abstract: The nematophagous fungus *Pochonia chlamydosporia* (Clavicipitaceae) can parasitize eggs of cyst (*Globodera* spp., *Heterodera* spp.), root-knot (*Meloidogyne* spp.) and false root-knot (*Nacobbus* spp.) nematodes. Its potential as a biological control agent has been the subject of numerous studies to understand the micro-ecological conditions, including the tri-trophic (*e.g.*, plant, fungus, nematode) and host-parasite relationships that allow the fungus to thrive in the soil and rhizosphere environment. *Pochonia* survives in soil in the absence of a nematode host and, although it behaves as a saprophyte, research evidence points to a physiological ‘switch’ from saprophytic to parasite stages triggered by nutrition. Fungal chlamydospore-based products for application to soil as an inoculum have been shown to be commercially viable. However, a

deeper understanding of the biology and physiology of the host-parasite interaction could provide new insights leading to an improvement in commercial production methodology.

Long-term effects of the application of organic amendments on soil fungal communities in pepper crops
M. A. Martínez, M. C. Martínez, J. Torres, J. Tello and A. Lacasa 81-84
Abstract: After methyl bromide phase out for soil disinfestation, the traditional agricultural practice of applying organic amendments into soil has been re-introduced. Organic amendments help in maintaining the fertility, soil structure and productivity of agriculture soils and preventing nutrient losses, but little is known about its long-term effect on soil fungal communities. The long-term effect of incorporating organic amendments of two different methods (biofumigation and biosolarization) has been studied by isolation of soil fungi as part of the microbiota in the beginning and in the end of the growing season during some years in different greenhouses. No amended soil and methyl bromide were considered as reference treatments. Results showed that these relatively new agricultural practices for soil disinfestations, based on environmentally-friendly techniques had similar results than the agricultural practice of soil disinfestation with methyl bromide, particularly at the end of the growing season.

Influence of management system in the natural suppressiveness of olive orchards soils to the soilborne plant pathogen *Verticillium dahliae*
Miguel Montes-Borrego, Juan A. Navas-Cortés, Rafael M. Jiménez-Díaz, Blanca B. Landa 85-89
Abstract: Olive (*Olea europaea* L. subs. *europaea*) is one of the most important crops in Spain with > 2.4 million ha. During the last two decades phytosanitary status of olive orchards is being threatened due to some diseases caused by soilborne pathogens, mainly Verticillium wilt (VW) caused by *Verticillium dahliae* (VD) which is steadily increasing both in severity and extension. The main objective of this study was to determine the effect of olive management system on the natural suppressiveness of olive orchards soils to VD and how this may be related with biological and functional indicators of soil quality. An *in planta* bioassay was developed for assessing the natural suppressiveness to VD of a collection of 93 soils using highly conducive conditions for VW development. Results showed a wide range of variation among the different soils in their ability to suppress VD, with 25.8, 49.5 and 24.7% of soils showing low, moderate and high suppressiveness according to the VW severity developed in the test plants growing in those soils compared to that developed in plants in sterile and pasteurized artificial soils. Canonical multivariate discriminant analysis is being used to identify physicochemical, functional or biological indicators that may be involved in the natural suppression to VD of those soils.

Cultivar-specificity of bacterial and stramenopile communities in the rhizosphere of *Olea europaea*
Henry Müller, Katja Drofenigg, Blanca B. Landa, Gabriele Berg 91-95
Abstract: The rhizosphere represents the interface between plant roots and surrounding soil with a highly active microbiota. The structure of associated microbial communities is affected by multiple factors including plant species and plant age but also soil characteristics and climate conditions. Here, we studied the influence of the olive variety on the bacterial community as well as on the presence of stramenopiles. Total DNA from rhizosphere samples of three Spanish and one Italian cultivar of *Olea europaea* from the same field site were extracted. Using single strand conformation polymorphism (SSCP) analyses, specific community profiles were generated and interpreted. Results revealed a cultivar-specific association with bacteria whereas the stramenopiles community appeared to be independent from the cultivar.

Stenotrophomonas: an ambivalent global player from the rhizosphere
Henry Müller, Christoph Schmidt, Muhammedali Alavi,
José Luis Martínez, Gabriele Berg 97-100

Abstract: The genus *Stenotrophomonas* is of high biotechnological, ecological but also medical interest due to the versatility of the different species. *S. maltophilia* and *S. rhizophila* are abundant inhabitants of rhizospheres of a broad phylogenetic range of plants. They are able to interact with eukaryotic cells, which results in positive effects on plant growth and health but also on negative impacts on human health. Host-microbe interactions are based on similar mechanisms. Only hypermutator strains (with higher mutation rates) are able to colonize clinical environments, while low mutation frequencies were particularly frequent among *S. maltophilia* strains from the rhizosphere. Only the non-pathogenic *S. rhizophila*, is a promising candidate for biocontrol and stress protection on plants.

Nematode community study in a germplasm bank of representative olive cultivars from the Mediterranean basin
Juan E. Palomares-Rius, Pablo Castillo, Miguel Montes-Borrego,
Henry Müller, Blanca B. Landa 101-104

Abstract: Olive is a key crop, both economically and socially in southern Spain. Although the interaction of plant-parasitic nematodes with free-living nematodes could play an important role in their control, it is not known how the olive genotypes may influence the rhizosphere environment and consequently the balance between plant-parasitic and free-living nematodes. The aim of this work was to study the nematode communities associated with rhizospheric soil and roots of a collection of 16 olive cultivars from a world olive germplasm bank at southern Spain and assess whether olive genotype determine differences in the nematofauna present in their rhizosphere. Classical nematological analysis, including soil nematode extraction, species counting and morphological identification was performed. In addition, total soil DNA was extracted from the same samples and used for analysis of genetic diversity of total nematode populations using Terminal Restriction Fragments Length Polymorphism (T-RFLPs) analysis of the 18S rDNA gene. Results showed that 24 taxa belonging to 8 genera (including *Criconemoides, Ditylenchus, Filenchus, Helicotylenchus, Merlinius, Paratylenchus, Tylenchus,* and *Xiphinema*) and 8 families (including Aphelenchidae, Belonolaimidae, Criconematoidae, Hoplolaimidae, Longidoridae, Sphaerularioidea, Tylenchidae and Tylenchulidae) of plant-parasitic nematodes were present, with one species (*Helicotylenchus digonicus*) being prevalent in all samples. There was no clear relationship between incidence or population density of each nematode species with the olive genotype. General nematode maturity index (MI) values were inconsistent between olive varieties and did not therefore distinguish differences in the levels of soil disturbance. However, plant-parasitic nematode index (PPI) was significantly modified by olive variety within the single field. In addition, T-RFLP analysis showed differences between olive cultivars, with samples associated with specific varieties showing higher diversity of nematode taxa.

Genetic diversity of *Xiphinema pachtaicum* and *Xiphinema index* populations Based on mitochondrial and nuclear DNA variation
Juan E. Palomares-Rius, Carlos Gutiérrez-Gutiérrez,
Carolina Cantalapiedra-Navarrete, Blanca B. Landa, Sofie Derycke
and Pablo Castillo 105-108

Abstract: The dagger nematodes *Xiphinema pachtaicum* and *X. index* are one of the most widespread and frequently occurring *Xiphinema* species co-infesting vineyards and other crops and natural habitats worldwide. The prevalence of *X. pachtaicum* and the importance of *X. index* as a virus-vector, together with the possibility of the latter being an introduced species and occupying a more restricted habitat than *X. pachtaicum* make them interesting nematodes for the study of population genetics. The primary objective of this study was to determine the genetic structure of *X. pachtaicum* and *X. index* populations using nine and seven populations, respectively, from different "wine of denomination of origin (D.O.) zones" in Spain and Sardinia

(Italy), by the study of the mitochondrial (cytochrome oxidase c subunit 1 or *COI*) and nuclear (D2-D3 expansion segments of 28S rDNA) markers. Both *Xiphinema* species showed low intra-specific divergence among *COI* sequences, ranging from 0.2% (1 base substitution) to 2.3% (10 substitutions) in *X. pachtaicum*, and from 0.2% (1 base substitution) to 0.4% (2 substitutions) in *X. index*. Population genetic structure with a significant variation among populations within D.O. zones and within populations were found for both species. Nevertheless, molecular differences among grapevine-growing areas were not significant. It is hypothesized that this genetic structure may have been originated by extensive and continuous grapevine cultivation in the sampled geographic areas, as well as inadequate sanitation practices during long periods. Results also demonstrated that the two DNA regions studied are suitable diagnostic markers for *X. index* and *X. pachtaicum*.

Pathogenic diversity of *Sclerotium rolfsii* populations infecting sugar beet crops From Mediterranean-type climate regions
Efrén Remesal, Rafael Jordán-Ramírez, Rafael M. Jiménez-Díaz, Juan A. Navas-Cortés 109-112

Abstract: The pathogenic diversity among and within 12 mycelial compatibility groups (MCGs) previously identified from a population of 459 *Sclerotium rolfsii* isolates infecting autumn-sown sugar beet crops in Mediterranean-type climate regions of Chile, Italy, Portugal, and Spain were determined to 11 economically important host crops. Across the tested plant species, MCG ii and iv were highly virulent, MCGs i, iii, ix and x moderately virulent and MCGs iv, v, vii, viii, xi and xii were low virulent. Similarly, across MCGs, chickpea and sunflower were highly susceptible, pepper, watermelon, cotton and tomato susceptible, broccoli, sugar beet, and melon, moderately susceptible, and wheat and corn were resistant. There was no MCG-host species significant interaction. Establishing the variability in virulence among *S. rolfsii* isolates is of importance for management of Sclerotium root rot diseases in crops that can potentially be affected.

Biosolarisation and grafting as a way to mitigate the selection of virulent populations of *Meloidogyne incognita* in pepper
Caridad Ros, Celia Martínez-Mora, Fulgencio Sánchez, Carmen María Lacasa, Maria del Mar Guerrero, Alfredo Lacasa 113-116

Abstract: Since the prohibition of methyl bromide as a soil fumigant to control *Meloidogyne incognita,* the use of rootstocks carrying resistance genes (Me1 and Me7) to mitigate the effects of nematode could be an alternative strategy. However, the continuous use of rootstocks in a given soil has led to the selection of some virulent populations. To prevent the selection of virulent *M. incognita*, are tested the combination of grafting on resistant rootstocks (carrying the gene Me7) and soil biosolarisation. A bioassay comparing the use of methyl bromide and biosolarisation in presence of grafted or ungrafted plants of pepper in two different sanitary conditions (presence or not of virulent nematodes) was performed in the region of Murcia, in Spain for 3 consecutive years. The results show that in highly contaminated situation the resistant rootstocks and the fumigation methods, alone or in combination may reduce but not prevent the attacks of virulent *Meloidogyne incognita* while in less infested area, the combination of biosolarisation with resistant rootstocks prevented the selection of virulent nematodes and may allow a sustainable pepper production.

Changes in virulence of populations of Meloidogyne incognita to grow pepper plants resistant to nematodes
Caridad Ros, Celia Martínez-Mora, Fulgencio Sánchez, Ana Cano, Alfredo Lacasa 117-121

Abstract: In the greenhouses of the Region of Murcia, pepper is being monocultured for more than two decades. *Meloidogyne incognita* is a major soil pathogen. Rootstocks harbouring resistance genes are an alternative to chemical control to mitigate the effects of the nematode. To determine the influence of the interaction between this new genetic material nature of some populations of parasitic nematodes to investigate the behavior of the reiteration of root stock carrying resistance genes to *M. incognita* particularized in the gene *Me7*. For four consecutive years, in four greenhouses, has followed the evolution of virulence of populations to this gene, comparing grafted plants carrying this gene with ungrafted plants susceptible to the nematode, grown in soil without disinfecting. We found differences in the interaction between plants grafted on rootstocks that carry *Me7* gene and nematode population, between greenhouse and between years. In three greenhouses we found nematode population infesting grafted plants (*Me7*) the same level that ungrafted plants, when these plants were grown in the same soil during 2 or 3 consecutive years. This didn't happen in other greenhouse.

Biocontrol of Fusarium Head Blight (FHB) in a multitrophic perspective

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Abstract: Mycobiota composition associated with DON-treated and not treated wheat haulms, buried in three different soils was evaluated. DON did not affect number and species profile of fungal isolates occurring in wheat debris after 7d of incubation. *F. oxysporum* isolated from haulms were confirmed for their identity by TEF sequencing and submitted to molecular typing by AFLP, in order to assess the effect of DON and soil type on genetic variability. Based on both analyses, *F. oxysporum* grouped into four main clades, independently from the soil of origin and the presence of DON in the baits. Isolates of *Pythium* spp., *F. oxysporum* (from the previous experiment) and *Trichoderma* spp. (from our collection) were tested for their ability to grow in presence of DON. Ten *Trichoderma*, 56 *F. oxysporum* and 2 *Pythium* isolates showed a statistically comparable growth rate in presence/absence of DON. Isolates not affected by DON were tested against *F. culmorum* and *F. graminearum* mycotoxigenic isolates. Effects of *Pythium* and *Trichoderma* against *F. oxysporum* were also evaluated. *Pythium* isolates showed a variable growth inhibition activity against *F. culmorum*, did not inhibit *F. graminearum* but parasitize macroconidia of both. *F. oxysporum* did not inhibit pathogens and was not affected by the other antagonists. Only three *Trichoderma* strains showed antagonistic and mycoparasitic activity against the pathogens and *T. gamsii* 6085, the best antagonist, was used in an *in vitro* competition test on natural substrates (wheat straw and rice), using qPCR to estimate the effect on pathogens' growth. Competition test on rice confirmed its ability to antagonize. On wheat straw, an extreme oligotrophic environment, *T. gamsii* 6085 seemed to develop very badly and did not affect the growth of both pathogens. This research aimed at selecting microfungi able to antagonize FHB agents and/or to compete with them for cultural debris in a multitrophic perspective.

Functional genomics analysis of the *Pseudomonas fluorescens* PICF7/ *Olea europaea* interaction: identification of ESTs during olive roots colonization by an endophytic biocontrol strain

Elisabetta Schiliro, Massimo Ferrara, Franco Nigro, Jesús Mercado-Blanco 129-133

Abstract: Within an integrated disease control strategy to promote long-term protection against Verticillium wilt of olive (*Olea europaea* L.), we aimed to elucidate the genetic and molecular processes taking place during the colonization of olive roots by *Pseudomonas fluorescens* PICF7, an effective biocontrol agent against VWO. SSH methodology and transcriptome analysis enabled the identification of putative genes differentially expressed such as several enzymes related to biosynthesis of plant hormones (i.e. lipoxygenase, chorismate synthase) and of phenylpropanoids compounds (i.e. phenylalanine ammonia lyase, cinnamyl-alcohol dehydrogenase). Interestingly, among the transcriptionally up- or down-regulated genes, diverse transcription factors implicated in plant signaling pathways for both biotic and abiotic stimulus response were also identified.

Influence of bird-induced soil fertility gradients on oomycete distribution in a threatened *Quercus suber* population
María S. Serrano, Paolo De Vita, Luis V. García, Cristina Ramo,
Cristina Aponte, Lorena Gómez-Aparicio, M. Esperanza Sánchez 135-139

Abstract: Cork oaks are considered keystone structures in the ecosystem of the stabilised sands of the Doñana National Park. These remnant big trees are threatened by nesting of colonial waterbirds (whose debris induce deep soil chemical changes) and by two pathogenic oomycetes, *Phytophthora cinnamomi* and *Pythium spiculum*. We analyzed the distribution of seven oomycete species in soils along a bird influence gradient. Canonical analysis revealed a significant relationship between the composition of the oomycete community and soil chemical variables closely related to bird debris accumulation (soil N and P contents). Some *Pythium* spp. were able to live in heavily fertilized soils, while pathogenic species did not thrive in that soils. A separate analysis showed that the presence and abundance of *P. cinnamomi* and *P. spiculum* in the rizosphere was significantly lower in trees with moderate or high bird influence. We concluded that 1) natural fertilization by bird faeces induced soil changes affecting pathogenic oomycetes behaviour; 2) as long as these bird-induced soil changes were no too extreme to cause dieback by themselves, they could prevent the spread and tree decline events caused by soil pathogens.

Functional metagenomics for accessing the riches in suppressive soil
Sara Sjöling 141

Abstract only

Bio-informatics tools for studying bacterial communities
Javier Tamames 142

Abstract only

Approaches to risks assessment after release of biocontrol agent *Penicillium oxalicum* in soil
Gema Vázquez, Antonieta De Cal, Paloma Melgarejo, Marta Mallorqui,
Maira Martínez-Alonso, Núria Gaju, Inmaculada Larena 143-148

Abstract: This study focuses on environmental risks assessment after application of *Penicillium oxalicum* strain 212 (PO212) in soil. PO212 successful survived until 365 days in soil under field conditions at a concentration of $10^{4\text{-}5}$ conidia g^{-1} soil with a $10^{2\text{-}3}$ viable colony forming units g^{-1} soil. PO212 dispersed as horizontal at distance around to 30cm from plant as vertically at depths of up to 10cm in soil. The PO212 application did not have any negative effect on soil fungi communities.

High shelf-life formulations for *Methylobacterium extorquens* DSM 21961, a microbial inoculant to enhance strawberry flavour
Markus Verginer, Henry Müller, Gabriele Berg 149-153

Abstract: *Methylobacterium extorquens* DSM 21961 is a promising flavour enhancing agent on strawberries. For the use as microbial inoculant, a method to produce and formulate the bacterium into a product with high-cell-densities and a long shelf-life is essential. We tested three different growing media and four formulation procedures: (i) a lyophilisate, (ii) a formulation in a xanthan matrix, (iii) an encapsulation in alginate with and (iv) without adaptation of the cells to storage conditions. After storage at six different temperatures and according to the calculated disintegration kinetics, the xanthan formulation as well as the adapted alginate formulation showed the best shelf-life. Strawberry plants were treated with these *Methylobacterium* formulations, but no statistically significant differences according to cell numbers on leaves were observed: DSM 21961 was shown to be established at a level of $\log_{10}$ 6.0 cells g^{-1} leaves monitored by strain-specific real-time PCR. Comparing all criteria (shelf-life, practicability, plant colonization, effect, expenses), the xanthan formulation can be suggested as optimal formulation procedure. For special applications like soil treatments, the alginate beads or the lyophylisate are appropriate formulations. Results open the way for the use of methylobacteria in agriculture.

Analysis of root-knot nematode early infection events in tomato colonized by the arbuscular mycorrhizal fungus *Glomus mosseae*
Christine Vos, Annemie Elsen, Bart Panis, Sebastien Carpentier, Christelle Guillier, Eliane Dumas-Gaudot, Dirk De Waele 155-158

Abstract: Arbuscular mycorrhizal fungi (AMF) are obligate root symbionts colonizing the majority of vascular plants on earth. The fungi receive photosynthetic carbon from their host, while the plant benefits from improved water and nutrient uptake, resulting in enhanced growth and survival. In addition, AMF have been shown to reduce the occurrence and impact of several soil pathogens including plant-parasitic nematodes on a wide range of agricultural crops. A well-established AMF-colonization is a prerequisite for AMF-induced biocontrol against nematodes, both locally and systemically. Apart from these observations however, the defense mechanisms involved have rarely been investigated for AMF-nematode interactions. Our experiments indicate that the presence of the AMF *Glomus mosseae* reduces root-knot nematode infection, and already influences the nematode *Meloidogyne incognita* in its early stage of plant infection. In order to understand the underlying mechanisms of this effect, we are undertaking an in-depth study by monitoring the AMF-plant-nematode interaction during early root-knot nematode infection.

Effect of traditional and new agricultural practices on pathogen and biological control agents populations and on soil suppressiveness
David M. Weller, Timothy C. Paulitz, Patricia A. Okubara, Dmitri V. Mavrodi, Kurtis L. Schroeder, Robert F. Bonsall, Olga V. Mavrodi, James A. Parejko, Linda S. Thomashow 159-167

Abstract: By 2050, there will be 9 billion people on earth to feed using the same amount or less land and water as is currently available for agricultural production. Currently about one third of all agricultural commodities grown worldwide are lost to diseases, insects and other pests. Soilborne diseases account for a significant amount of those annual losses in food production, and crop plants often lack resistance to soilborne pathogens. Farmers will need to increase production, but must do so using less pesticides and more sustainable cropping practices. This paper discusses how changes in agricultural practices that are needed to enhance sustainability in 21st century wheat production also impact populations of soilborne pathogens and diseases, disease suppressive soils and populations of biocontrol agents. We focus on the Pacific Northwest of the USA, and more specifically on wheat production in the state of Washington. Changes to more sustainable agricultural practices such as reduced tillage have led to an increase in the incidence of soilborne diseases but new agricultural practices as well as techniques in precision agriculture and molecular detection of soilborne pathogens are finding solutions to disease problems. Indigenous biocontrol *Pseudomonas* spp. such as those producing 2,4-diacetylphloroglucinol and phenazine-1-carboxylic acid have a role in natural suppression of disease, but their populations and role in disease suppression are also affected by changes in agricultural practices.

Impact of induced systemic resistance on the bacterial microbiome of *Arabidopsis thaliana*
Rogier F. Doornbos, L. C. Van Loon, Peter A. H. M. Bakker 169-172

Abstract: Induced systemic resistance (ISR) has been described for several strains of fluorescent pseudomonads in a variety of crop plants and is effective against a wide range of pathogens. Using the model plant *Arabidopsis thaliana*, progress has been made in understanding signal transduction pathways involved in induced resistance. Perception of ethylene and jasmonic acid is important for ISR. The traits of *Pseudomonas* spp. that can trigger ISR appear to be diverse, and include iron regulated metabolites, antibiotics, and lipopolysaccharides. Since pathogen growth is restricted on plants that are in the state of ISR, we hypothesized that the indigenous microflora could also be affected by ISR. Using cultivation dependent techniques, effects of plant defense signaling on the total bacterial and the *Pseudomonas* spp. microflora of *Arabidopsis* were studied and related to susceptibility of *Arabidopsis* genotypes to bacterial speck caused by *Pseudomonas syringae* pv. *tomato*.