Barley soil borne mosaic viruses: Identification of predominant viruses affecting yield and malting quality, in order to orientate breeding towards a sustainable resistance

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12th European Foundation for Plant Pathology (EFPP)

& 10th French Society for Plant Pathology (SFP)

CONFERENCE

DEEPEN KNOWLEDGE IN PLANT PATHOLOGY FOR INNOVATIVE AGRO-ECOLOGY

BOOK OF ABSTRACTS

May 29 - June 2 2017

Dunkerque - Malo-les-Bains - FRANCE


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OPENING SESSION

Opening lecture 1

Global change is a challenging parameter for plant pest risk assessment.

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Global change refers to planetary-scale changes in the Earth system which includes human society. Global change encompasses climate, land use, biodiversity, trade of plant and plant parts and has a strong and well defined impact on plant health despite the short time frame under consideration. Emerging plant diseases present many serious issues for human well-being, whether in agricultural, forestry, environmental or regulatory arenas. The temporal and spatial scales of plant disease emergence are defining features related to local, national and global drivers. These include the increases in global trade, of course, but also the introduction of novel crop, changes in production systems, interactions occurring at the landscape level and the impact of climate change. Agroecology is a one of the drivers that raise questions about the predictability of emerging plant diseases
Pest risk analysis (PRA) is the process used by National Plant Protection Organizations (NPPOs) as the technical justification for phytosanitary measures. PRA is defined by the International Plant Protection Convention (IPPC) as "the process of evaluating biological or other scientific and economic evidence to determine whether a pest should be regulated and the strength of any phytosanitary measures to be taken against it." The process requires a risk assessment to characterize the risk and risk management to determine appropriate measures. PRAs are mainly performed in the context of the international trade. An introduced pathogen in one region that leads to an emerging disease may have been endemic, widespread and sometimes cryptic in another. Besides, it may be re-emergence of a disease which had disappeared because of modifications in production systems. Genetic changes through hybridization can lead to host shifts and adaptations. Less often, the emerging disease may be caused by a pathogen that is hitherto new to science.

Keywords: emerging diseases, Pest risk analysis, IPPC

Opening lecture 2

Valuing Biodiversity and Biotic Interactions for Crop protection in Agroecology

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The intensive agriculture initiated after the second world war is now considered as being unsustainable because of the erosion of resources (e.g. soils, biodiversity, fossil energy) and the high use of inputs (pesticides, fertilizers, water) with possible deleterious effects both on the environment and on the quality of agricultural products. Accordingly, an increasing interest is given to agroecology with the development of cropping systems which value biodiversity and biotic interactions in agroecosystems. Various illustrations and prospects will be given on the role of biodiversity and biotic interactions in (i) soilborne disease suppression, (ii) the balance between the disservices and services provided by weeds, (iii) keeping the level of pests below a harmfulness threshold. This presentation will encompass the evaluation of (i) the beneficial effects on crop protection of biodiversity (level and composition) and biotic interactions (including trophic networks), and of (ii) the enhancement of these effects by appropriate crop management (including the choice of plant genotypes) at the plot and landscape levels, allowing the decrease of pesticide use while keeping the productivity high enough.

Session 1. FROM PLANT-MICROBE INTERACTIONS TO INTERACTIONS WITHIN PHYTOBIOMES (Salle Jean Bart)

Keynote lecture 1

Phytobiome ecology: developing novel approaches for sustainable disease management

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Microbes and plants exist within complex networks of interacting plant and microbial species, the phytobiome. Our work explores the interacting roles of plant community diversity, plant host, and microbial species interactions in determining the pathogen-suppressive potential and composition of soil microbiomes, and the consequences for plant productivity. Using culture-based approaches, we found that rhizosphere *Streptomyces* associated with the same plant host were significantly more pathogen-suppressive when the host grew in monoculture vs. within a high-diversity plant community. In contrast, populations of *Streptomyces* in the rhizosphere of plant hosts growing in high-diversity communities were more niche-differentiated than populations associated with the same host in monoculture. These data suggest that plant community diversity plays a critical role in determining the likelihood of antagonistic arms race coevolution vs. niche differentiation among sympatric soil populations, with significant implications for plant disease suppression. Amplicon sequencing of rhizosphere communities associated with different plant hosts provide insights into non-cultured taxa associated with disease suppression. In total, our work illustrates how diffuse networks of species interactions over diverse spatial scales contribute to determining the pathogen-suppressive and plant growth-promoting potential of indigenous soil microbes, and suggests specific crop management approaches targeting species interactions that offer potential for sustainable disease control.

Keywords: phytobiome, disease suppression, coevolution, suppressive soil, antagonist, *Streptomyces*

Keynote lecture 2

Individual-based ecology of the phyllomicrobiome

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The interactions of plant-associated microorganisms among themselves and with their host are complex and take place across a wide range of spatial scales. There is a limited understanding of the types, mechanisms, and outcomes of these interactions at the lowest scale, i.e. at the level of individual microbes, and how these interactions link to observations at higher-level spatial scales. Here, I will explore recent progress that has been made in the field of individual-based ecology of the phyllomicrobiome, i.e. the plant leaf surface as a habitat for microorganisms. I will present examples from our own work to highlight the value of the individual-based approach as well as the use of new research tools that allow the single-cell interrogation of a microbe's experience of its environment, the deconstruction of leaf surface complexity to understand the impact of microscale factors such as leaf surface topography, and the *in silico* simulation and prediction of individual-based interactions in a heterogeneous environment.

Keywords: phyllosphere, individual-based ecology

Session 1
Oral 1

Plant-microbe interactions in strawberry grown in sustainable and disease suppressive substrates

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Peat based substrates are not sustainable and often fail to support biological control, even when inoculated with biocontrol agents. Locally produced plant fibres may allow for partially replacing peat in substrates. Moreover, they may easily be colonized by microorganisms and in this way promote the installation of biological control agents resulting in disease suppressiveness.
Strawberry pot trials were conducted using miscanthus straw as a 20% v/v amendment in peat. The miscanthus straw was extruded and pre-colonized with the biocontrol fungus *Trichoderma harzianum* (MSEXTRI treatment). Strawberry plants grown in this treatment were significantly less susceptible to *Botrytis cinerea* inoculated on the fruits as compared to 100% peat and peat with 20% v/v extruded miscanthus straw (MSEX treatment). Both bacterial and fungal rhizosphere communities were studied using 16S rDNA V3-V4 and ITS2 metabarcoding, respectively. The specific presence of *Trichoderma* in the rhizosphere was studied using platings on semi-selective medium. The strawberry defence response was studied through gene expression analysis.
Adding extruded miscanthus straw significantly reduced the fungal diversity in the strawberry rhizosphere. Surprisingly, in the MSEXTRI treatment, the relative abundance of the fungal *Humicola* spp. was highly increased as compared to the 100% peat and MSEX treatment, whereas the relative abundance of the *Trichoderma* spp. was low in all treatments. *Humicola* spp. are known for their potential in lignocellulose degradation and induced resistance. Plating on semi-selective medium showed that only the strawberry roots of the MSEXTRI treatment were consistently colonised by *Trichoderma*. Bacterial communities maintained their diversity and did not exhibit significant changes, although some genera shifted in relative abundance between the treatments. The strawberry plants grown in the MSEXTRI treatment showed an up-regulation of defence genes *Fachi2-1*, *Fachi2-2* and *FaPAL* as compared to the other treatments. This might be correlated with the observed changes in the rhizosphere microbiome and disease suppressiveness.

Keywords : bacteria, fungi, miscanthus, metabarcoding, gene expression, *Trichoderma*

Session 1
Oral 2

Oxylipin communication between the fungal endophyte *Paraconiothyrium variabile* and the phytopathogen *Fusarium oxysporum*.

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It is more and more evident that in order to study plant pathogen infection processes, one has to take into account the presence of third protagonists, the endophytic and epiphytic microbiota. How fungal endophytes communicate with invading plant pathogens, the microbiota and the host plant is the topic of our research project.

We have at our disposition a range of isolated fungal endophytes from the conifer tree *Cephalotaxus harringtoni*[1] and in particular, a foliar species, *Paraconiothyrium variabile* (Dothidiomycetes*).* Initial research on this endophyte indicates antagonistic activity towards phytopathogens, inhibition of mycotoxin production in *F. oxysporum*[2] and plant metabolite biotransformation to the advantage of the endophyte[3]. During the competition between *P. variabile* and *F. oxysporum*, two oxylipins, 13-keto-9,11-octadecadienoic acid (13-KODE) and 13-hydroperoxy-9,11-octadecadienoic acid (13-HpODE) are overproduced, which is accompanied by a decrease in beauvericin secretion, one of the most potent mycotoxins of *Fusarium* species and a virulence factor on infected plants.

To elucidate the role of the two oxylipins in the fungal antagonistic interaction, we identified and cloned two lipoxygenase genes (*Pvlox1* and *Pvlox2*) of *P. variabile potentially involved in the biosynthesis of* 13-KODE and 13-HpODE. We expressed the two *Pvlox* genes in *Escherichia coli* and currently study the biochemical activity of the PvLOX1 and PvLOX2 enzymes. In a complementary approach, we study the expression patterns of the two *Pvlox* genes in *P. variabile* during its antagonistic interaction with *F. oxysporum*. To expand the research on an *in planta* tripartite model system, we started inoculation tests on *Arabidopsis thaliana* plants to follow *P. variabile* infection microscopically and test its influence on the plant fitness.

1. *Langenfeld, A. et al. (2013), Fungal Biol.* ***117,*** *124–136*
2. *Combès, A. et al. (2012), PLoS ONE* ***7,*** *e47313*
3. *Tian, Y. et al. (2014), Phytochemistry* ***108,*** *95–101*

Keywords: Fungal endophyte, oxylipin signalling, lipoxygenase, mycotoxin, *Fusarium oxysporum*, *Arabidopsis thaliana*

Session 1
Keynote lecture 3

Viruses in the Phytobiome: Abundance and Ecological Roles.

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We have analyzed plant viruses in a biodiversity hotspot in northwestern Costa Rica with about 10,000 species of native plants, and a low diversity natural area in the United States: the Tallgrass Prairie Preserve. We find many viruses that are distantly related to known viruses, an equal or greater number of completely unknown viruses, and a few known viruses. Persistent viruses are the most common, and appear to have very long relationships with their hosts with nearly 100% rates of vertical transmission. Acute viruses are found in each study site, some with wide-spread infections, but known are linked to any symptoms. Mixed infections are also common.

Members of the family *Partitiviridae* are the most common viruses found in both studies of wild plants, and are also very common in crops. We are exploring the impacts of these viruses on crop plants using Japapeña peppers, which are always infected with *Pepper cryptic virus* 1. Beneficial effects of virus infection will be discussed, including the implications for agriculture.

Session 1
Oral 3

Lipopeptides involved in the rhizosphere fitness of *B. amyloliquefaciens* biocontrol strains

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Bacilli are among the genera most commonly identified in the bacterial rhizosphere community associated with plant roots. Interestingly, some isolates from particular species such as *B. amyloliquefaciens* clearly represent beneficial partners for the host plant based on their potential to combat phytopathogens and/or to directly promote growth and are promising candidates for the development of microbial alternatives to chemical pesticides or fertilizers. Such plant protection effect is associated with their capacity to form a wide array of biocontrol secondary metabolites (BSM) retaining antimicrobial and host immunity eliciting activities. However it also primarly relies on their potential to establish and persist in the rhizosphere at threshold populations necessary to form relevant amounts of these BSM.

We will provide an overview of bacterial traits involved in such rhizosphere fitness and present our recent works showing how some unsuspected plant molecular patterns may also impact the phenomenon. We combined imaging and other MS techniques with genetic approaches to show that *B. amyloliquefaciens* can boost the synthesis of a particular lipopeptide-type antibiotic (surfactin) upon perception of some polysaccharides present in the root cell wall. A fast accumulation of this multifunctional antibiotic in the root environment is actually of clear benefit for both partners. On one side, it favors the ecological fitness of the bacterium by improving motility, biofilm formation and early root tissue colonization and on the other side, the lipopeptide rapidly reaches threshold concentrations necessary for triggering some immune-related molecular responses in the host plant leading to a significantly lower susceptibility to phytopathogen ingress. These data provide new insights into the chemical dialogue between the two organisms and more broadly, into the subtle molecular mechanisms driving the multitrophic plant-microbe interactions in the rhizosphere environment. It also opens the door to further investigations for better understanding how bacilli and other rhizobacteria can sense their environment and natural plant hosts.

Keywords: cell wall polymers, root exudates, lipopeptides, surfactin, plant immunity

Session 1
Oral 4

Agrobacterium opines: from tumor markers to microbial ecology tools

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Opines are low molecular weight molecules that are typically found in crown gall tumors and hairy root hyperplasia incited by members of the *Agrobacterium* and *Rhizobium* genera. Opine presence in the transformed cells creates an environment favorable to the pathogen, promoting both its growth and the dissemination of the pathogenicity.

Aside from their role in *Agrobacterium* ecology, opines have been used for some 20 years to investigate how plant exudation shapes the diversity of the associated microbiota. Transgenic plants producing opines have been produced and installed in nonsterile “real” soil. Generally speaking, while the carrying capacity of the plants and the concentration of members of several microbial populations remained unaffected, a drastic stimulation of the opine-degrading community was observed, whatever the opine produced, the plant genus and the soil characteristics. However, the taxonomic position of the members of the opine-degrading communities varied as a function of the opines produced by the plant. This type of simple experiment allowed us to verify the validity of the “rhizosphere effect” and to demonstrate that the composition of the associated microbiota may depend on plant exudates that may not be produced anymore at the time of the analysis.

More recent experiments based on a similar experimental pattern were set up to analyze more finely the changes that affected the plant microbiome, using 16rRNA analyses and next generation sequencing technologies (NGS). Using both transgenic plants that produced two different concentrations of opine and an artificial exudation device, we demonstrated that the amount of carbon affected by the modification of the exudation is a critical parameter involved in the reshaping of microbial populations. We also demonstrated that root-associated bacteria may have developed two different strategies to colonize the plant surface, i.e. copiotrophy (the ability to use a wide range of substrates) and oligotrophy (the ability to utilize very efficiently only a limited number of substrates).

Keywords: Agrobacterium, opines, NGS, root colonization, rhizosphere, microbiota

Session 1
Oral 5

Effect of plant mycorrhization on the rhizospheric microbiome structure in a context of polluted soils

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Polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF), collectively termed dioxins, are the most toxic group of persistent organic pollutants (POP). Due to their structural stability, derived from two aromatic rings containing one to eight chlorine atoms, PCDD/F are widespread in the environment and are considered to be harmful both for environment and human health (immunotoxicity, neurotoxicity, carcinogenicity). In the current work, Arbuscular Mycorrhizal Fungi (AMF) –assisted phytormediation is proposed to restore aged PCDD/F polluted soil. This technique is environmental friendly, potentially cost effective and not altering the soil matrix. Moreover, AMF improves the phytotechnology effectiveness by providing protection to the plants against the pollutant toxicity and by enhancing pollutant dissipation. The contribution of mycorrhizal inoculum addition on the ability of four plant species (Clover, Alfalfa, Ryegrass and Tall fescue) to grow and to dissipate PCDD/F was assessed after 20 weeks of microcosm culture. Our findings showed that the four plant species were able to grow on the contaminated soil and the addition of arbuscular mycorrhizal inoculum provides a high colonization rate despite the presence of PCDD/F in the soil at the concentration of 200ng/kg. The vegetation led to PCDD/F reduction of 38.6 ng/kg with the Tall fescue. This dissipation was found to be due to the stimulation of the microflora evaluated through microbial enzymatic activities (hydrolases and dehydrogenases) and specific bacterial lipid markers (Phospholipid Fatty Acids). In parallel, the evolution of structure and diversity of microbiomes refers to the whole community of bacteria, archaea and fungi after phytoremediation of sterilized or non-sterilized soil were examined. It was showed that bacterial and fungal community structures were significantly affected by arbuscular mycorrhizal inoculation, vegetation and the initial microbial state of the soil.

Keywords: dioxins, phytoremediation, arbuscular mycorrhizal fungi, sterilization, vegetation, rhizosphere microbiome.

Session 2. SPACE-TIME AND MULTI-SCALES APPROACHES: DIAGNOSTIC, EPIDEMIOLOGY AND ECOLOGY IN THE FIELDS

Keynote lecture 4

Non-invasive sensors and digital technologies for characterizing plant-pathogen interactions- applications for precision crop protection and plant phenotyping

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The detection and identification of plant diseases is a fundamental task in sustainable crop production. An accurate estimate of disease incidence, disease severity and negative effects on yield quality and quantity is important for crop production, horticulture, plant breeding or fungicide screening as well as in basic and applied plant research. Remote and proximal sensing techniques have demonstrated a high potential in detecting disease and in monitoring crop stands for infected plant areas [1]. Most promising sensor types are thermography, chlorophyll fluorescence and hyperspectral sensors. This variety of sensor systems available provides high resolution data of agricultural crop stands or single plant organs and can constitute the basis for an early detection and identification of plant diseases. Particularly hyperspectral imaging of diseased plants offers insights into processes during pathogenesis. Fungal leaf pathogens influence both, the structural characteristics and the leaf chemistry which in turn is reflected in the optical properties of plants. By hyperspectral imaging and subsequent data analysis routines it was possible to realize an early detection, identification and quantification of different relevant plant diseases [2]. However, the complex web of factors, influencing optical characteristics of diseased plants remain poorly understood. Additionally scientist working with these technologies regularly confront the problem of dealing with massive, high-dimensional, and temporal observations, posing a challenge in scalability. To utilize the full potential of these highly sophisticated, innovative technologies and, complex data for precision crop protection or plant phenotyping, a multi-disciplinary approach— including plant pathology, engineering, and informatics—is required [3].

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[3] Wahabzada, M., Mahlein, A-K., Bauckhage, C., Steiner, U., Oerke, E-C., Kersting, K. (2015): Metro maps of plant disease dynamics - automated mining of differences using hyperspectral images, PLOS One 10(1): e0116902

Session 2
Oral 6

Local and landscape effects on European grapevine moth and Grape powdery mildew in vineyards

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Agricultural areas are characterized by a heterogeneous landscape which includes a variety of agricultural crops alongside natural areas, forests, built areas etc. The landscape and the environment have an impact on the dynamics of pests and pathogens in space and time. Until recently, studies were based on small number of observations collected in the field or from the laboratory. Due to the complexity of the biological system, it is difficult to define the importance of the variables in controlled trials or in small extent studies and to conclude reliable relationships. Agricultural research is increasingly becoming a data-intensive science, relaying on massive amount of data collected in the field. In this study we examined predictors of the distribution of two important pests in vineyards- European grapevine moth *(Lobesia Botrana*) and Grape powdery mildew (*Uncinula necator*) at both local and landscape scales. We used monitoring data collected in 220 commercial vineyards. In the local scale we found that the most influential variables, for both pests, were the grower and the cultivar. For the landscape scale, we characterized the landscape context around the plots in 10 nested circles ranging from 100 to 1500 m in radius. We obtained significant positive correlations between European grapevine moth incidence and the total area of vineyards (peaking at 100 m), orchards (200 m), forest areas (1500 m) and built areas (1500 m). In contrast, Grape powdery mildew was not correlated to landscape context. These results demonstrate that landscape structure have direct effects on pests. These effects depend on both the species and the scale. Understanding the effects of landscape context can improve our understanding regarding pest biology and offers powerful information for future research about dispersal-related characteristics of pests and can improve pest control management.

Keywords: land-use, *Lobesia Botrana*, *Uncinula necator*

Session 2
Oral 7

The role of infected flower petals and leaves, and precipitation in predicting Sclerotinia stem rot of oilseed rape

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Sclerotinia stem rot (SSR) is an increasingly important disease of oilseed rape in many parts of the world. *Sclerotinia sclerotiorum*, known as the causal agent of SSR, has an extremely wide host range including many vegetable crops and weeds. The fungus produces sclerotia, which can survive dormant in the soil for many years. Sclerotia germinate under wet conditions and discharge ascospores from apothecia. The spores are thought to infect their rapeseed host via flower petals caught in leaf axils. Availability of resistant varieties is limited, hence rotations as well as fungicides are the most common ways to control the disease. Aligning fungicide applications with the actual need for control has led to the development of several disease risk prediction models. However, the accuracy of prediction for most of these models is not satisfactory. The objective of our work was to investigate some of the key factors in the pathogen's life cycle: the importance of precipitation and the role of flower petals.

We grew spring oilseed rape (*Brassica napus*) cultivar 'Mosaik' in the greenhouse until flowering and placed them in the field. Plants were exchanged every seven to ten days during the growing season with healthy flowering plants. After being exposed to field conditions, the plants were covered with plastic bags, brought inside and incubated for two to three weeks before visual assessment for SSR infection. We repeated this experiment over four years and at three different locations in Southeast Norway. Local weather stations provided data on precipitation, temperature and relative air humidity. Neither precipitation during exposure in the fields, nor precipitation one and two weeks before exposure time, correlated significantly with SSR infection of the plants.

Flower petals and green leaves of rapeseed plants were collected from nine different field trials over two years in Southeastern Norway and tested with qPCR for presence of *S. Sclerotiorum*. Severity of SSR infection in the field trials were assessed at harvest and correlated with percentage of positive leaf and/or petal samples tested. There was no correlation between SSR severity in the field and percentage of positive leaf or petal samples.

Several SSR risk models use precipitation and flowering as determining factors for SSR infection. Our studies indicate that precipitation is not as predictive for infection as generally thought. Soil humidity or leaf wetness might be more important factors in assessing the risk for SSR infection and spread. Flower petals play an important role in the SSR epidemic, but the relationship between petal infection and SSR infection appears not to be as direct as previously indicated.

Keywords: *Sclerotinia sclerotiorum*, sclerotinia stem rot, rapeseed, canola, oilseed rape, epidemiology, infection periods.

Session 2
Oral 8

When a plant pathogen runs down a river: population genetics of the poplar rust epidemics in the Durance River valley.

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Tracking pathogen movement, identifying source populations and understanding environmental factors, including human activities, that influence pathogen spread are central tasks in disease ecology. Here we focus on the spread of a plant pathogen in a wild pathosystem. Every year we monitored an annual epidemic of the European poplar rust fungus, *Melampsora larici-populina*, in the Durance River valley, in the French Alps. This valley is particularly well suited for the study of recurrent biological invasions: the need of an alternate host plant (larch) to perform its sexual reproduction restricts the resident pathogen population upstream the river, in a poplar-larch sympatry area. Then, a clonal epidemic phase spreads downstream the valley during five months along a ca. 200 km natural riparian stand of black poplar, *Populus nigra*. This landscape also includes a few cultivated stands with poplars carrying qualitative resistances, thus exerting a peculiar selection pressure on pathogen populations. In this study we used epidemiology and population genetics tools to (*i*) sort *M. larici-populina* individuals according to their wild or cultivated origin, (*ii*) to describe the spread of the epidemic on the wild poplar stands, (*iii*) to assess the evolution of the genetic composition of the pathogen populations along the epidemic wave, and (*iv*) to assess the evolution of life history traits during the epidemic. These results are discussed in the light of recent studies focusing on the relative effects of demographic and selection events on the evolutionary changes accompanying biological invasions.

Keywords: disease ecology, landscape epidemiology, colonization, dispersal, range expansion, wild pathosystem

Session 2
Oral 9

Spatiotemporal patterns of *Phytophthora megakarya* infections in newly established cacao plantations in Cameroon

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Studying spatial and temporal plant disease dynamics helps to understand pathogen dispersal processes, a necessary undertaking in epidemiology in order to improve disease control recommendations. In this study, plantations devoid of primary inoculum of *Phytophthora megakarya* (causal agent of cacao black pod disease), upon establishment in 2006, were monitored for presence of disease on a weekly basis from 2009 to 2016. Isolates of *P. megakarya* collected in these plantations were genotyped with 14 SSR markers. Ripley's K functions were used to characterize spatial disease dynamics. The univariate K-function was used to describe spatial disease patterns and the bivariate K_{12}-function was used to describe the relation between healthy and diseased cacao trees. Disease distribution maps show aggregated disease patterns in all plots. The K-function confirmed these results although it was not significant in all patterns, probably due to a limited number of diseased cocoa trees. Healthy and diseased cacao trees were mostly negatively correlated, indicating that cacao black pod disease dispersal is a clustered process preferentially affecting neighbors of already infected trees. Based on observations it appears that occurrence of black pod disease is not a complete randomized process. The neighboring environment can greatly influence disease dispersal processes. For instance, closeness to already infected cacao plantations can favor dispersal of disease propagules while the presence of a river can increase the disease incidence and pathogen diversity. According, to the results, black pod disease is mainly spread over relatively short distances. Isolation of newly established cacao plantations from infected ones appears therefore to be an effective approach to control of black pod.

Keywords: *Phytophthora megakarya*, first infections, spatial pattern, temporal evolution, cacao

Session 2
Oral 10

Dissemination of *Xanthomonas fragariae* in a strawberry field crops

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Xanthomonas fragariae (*Xfr*) is the causative agent of angular leaf spot in strawberry, a quarantine organism in Europe (EPPO A2). The dissemination of the pathogen in symptomatic strawberry fields by machineries, splash water and aerosols was studied.
A rotary mower was used to clip leaves of symptomatic plants. The cutter blades of the machine became heavily contaminated. Use of these contaminated machine resulted in (symptomless) infections of plants initially free of *Xfr*, even at 10 m distance from the symptomatic plants. Cleaning of the mower with a water hose was not sufficient to remove the pathogen.
Spread of *Xfr* from symptomatic plants by splash water during overhead irrigation at moderate wind, resulted in a spread over a distance of at least four meter. At windless weather conditions and light rain a similar minimum distance of spread was found but bacterial densities recovered were lower. An exponential decrease of *Xfr* density was found with distance from the inoculum source.
In artificially created aerosols, *Xfr* could be collected by an air sampler up to a distance of 100 m from the source and those created by mowing a symptomatic crop up to a distance of minimally 25 m. Aerosols released by a sprayer, carrying in total ca. 10^{11} cfu of *Xfr* was able to cause infections of strawberry plants up to distance of at least 10 m of the sprayer.
It is concluded that there is a considerable risk of spread of *Xfr* from symptomatic plants by machines and wind-driven aerosols within and between fields. Splash dispersal during rain or overhead irrigation can result of spread *Xfr* within the field.

Keywords: Angular leaf spot, machines, aerosols, air sampler, disinfection, splashing water

Session 2
Oral 11 (invited lecture)

Seasonal ascospore release by the wheat eyespot pathogens *Oculimacula yallundae* and *O. acuformis* in the Northwest USA

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Eyespot disease of wheat is caused by *Oculimacula yallundae* (OY) and *O. acuformis* (OA). Apothecia of OA and OY were first reported in commercial wheat fields in the U.S. Pacific Northwest (PNW) in 2003 and 2014, respectively, but their role in eyespot epidemiology remains unknown. Population genetic studies of OA and OY in the PNW concluded that both species were undergoing sexual reproduction, but its frequency and role in the disease cycle are not known. A previous ascospore trapping study conducted in inoculated field plots demonstrated that ascospore release occurred in spring and autumn, with more spores trapped in spring. In this study, ascospore trapping was conducted near noninoculated wheat fields to determine whether and when ascospores were present. Ascospores were collected with Burkard volumetric spore samplers during the 2014-15 and 2015-16 crop seasons at four locations near Pullman, WA (S15, S16, PP15, and PC16). Sticky tapes were collected weekly, examined microscopically, and then DNA was extracted and RT-PCR used to quantify DNA, which was converted to number of ascospores.

Ascospores of OA and OY were present in both crop seasons, with more spores collected in 2015-16 than 2014-15 at all locations. The greatest release of ascospores occurred in autumn at three locations (S15, PP15, and PC16) and was equal in autumn and spring at the other location (S16). More ascospores of OY than OA were collected in both years at all locations; based on DNA content, OY was 2.3 to 4.8 times more frequent than OA. It is not clear why more ascospores were present in 2015-16, but may be related to above average temperatures during spring 2016. It is clear from these data that ascospores are present during the autumn, which is the primary infection period, and may help explain the high genotypic diversity observed in previous studies.

Keywords: wheat, eyespot, epidemiology

Session 2
Oral 12

A possible evolutionary response of leaf fungi pathogens of cereals to changes in fertilization level.

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We use a model of life history evolution of crop fungal pathogens aiming to understand how they adapt to fertilization. We focus on a single pathogen life history trait, the latent period, important for pathogen resource allocation strategies and canopy colonization. We implement three fertilization scenarios corresponding to major effects of nitrogen fertilization on cereals: increase in (i) metabolites in leaves, (ii) leaf lifespan and (iii) canopy leaves density. We find that spores production increases with higher fertilization. We use two fitness measures to identify putative evolutionary responses of latent period to changes in fertilization. When fitness is defined as annual spores production, we predict a positive correlation between the optimal latent period and fertilization. By contrast, the model predicts a negative relationship between the optimal latent period and fertilization when fitness is defined as the within-season exponential growth rate of the pathogen. We hypothesize that if the critical step in the co-dynamics of multiple strains is the colonization of new fields then the limiting step will likely be the amount of spores in next year's inoculum, which will influence the early steps of epidemics. In that case, the strain that produces most spores over a season is likely to win the competition, and evolution should maximize annual spores production. If, on the other hand, the competition between strains is mainly experienced by within-season competition for healthy crop tissue, then the rate of spread through the canopy will be critical. We predict that reducing fertilization will change the aggressiveness profiles of different biotrophic fungius species or strains in the field.

Keywords : Epidemiology, Fertilization, Latent Period, Life History Theory, Resource-based Model, Structured-populations Model

Session 2
Oral 13

Genetic diversity and population structure of *Zymoseptoria tritici* in France at different spatial scales

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Zymoseptoria tritici is the most frequently occurring and damaging foliar pathogen on wheat crops in Europe, especially in France, where environmental conditions are suitable for disease development. More than 1500 isolates of this fungus, isolated in France over a ten-year period (2005-2015) and at different spatial scales (country, Hauts-de-France region, field, 30 cm^2 square, plant, leaf and lesion), were characterized using SSR markers and mating type idiomorphs in order to gain insight into fungal population genetic features. Results revealed overall high and similar levels of genic and genotypic diversities at the country, region and field scales, but diversity values gradually decreased from the field to finer scales, such as 30 cm^2 square, plant, leaf and lesion. Bayesian and non-Bayesian statistical analyses revealed significant structuration of the global French population into three genetic clusters, and the Hauts-de-France population into three sub-clusters, all distributed according to their geographical origin. However, no genetic differentiation was found at finer scales. Both mating types were found at equal frequencies at the country, region and field scales and found to co-occur together at all other investigated scales. This study revealed a high potential for sexual recombination and genetic diversification for *Z. tritici* in France, as well as a structuration of its populations at largest scales, in accordance with local adaptation to agro-climatic conditions facilitated by high fitness degree of the pathogen.

Key words: *Zymoseptoria tritici*, genetic diversity, population structure, SSRs, mating types.

Session 2
Oral 14

Leaf blotch on durum wheat in France: population genetics, host specialization and fungicide resistance

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Leaf blotch, caused by *Zymoseptoria tritici* and *Parastagonospora nodorum*, is a major disease complex of wheat worldwide. In France, these diseases have been reported to cause severe yield losses in bread wheat, most of which were due to *Z. tritici*. In contrast, our knowledge of the fungal species causing leaf blotch on durum wheat is limited whereas the occurrence of epidemics has been increasing in recent years. We recently conducted a 3 years survey of leaf blotch on durum wheat and bread wheat in four main French growing regions. More than 1100 isolates of *Z. tritici* were sampled from naturally infected fields (treated and untreated fields) of bread and durum wheat and genotyped using 12 microsatellite markers. The frequencies of occurrence among sites of the two fungal species in leaves were also evaluated using microbiological isolation methods and qPCR detection. Lastly, the level of host specialization and aggressiveness of a subset of *P. nodorum* and *Z. tritici* isolates were also determined by cross inoculation experiments on a panel of bread and durum wheat varieties. Based on molecular test results and isolation assays, *Z. tritici* was detected in all French regions on durum wheat and bread wheat. *P. nodorum* was detected on durum wheat in most French regions but was quantified only at low levels by qPCR on bread wheat. French durum wheat cultivars were highly susceptible to the French *P. nodorum* isolates tested. The genetic diversity of *Z. tritici* was structured by host and location of sampling. Inoculation experiments pointed out specialist, generalist and maladapted strains of *Z. tritici* in relation with population genetic groups. No differences between *Z. tritici* bread wheat populations and durum wheat populations were observed for fungicide resistance. These results show that control of leaf blotch on durum wheat, in France, needs to take into account the presence of both species and growing regions. These results may help to adapt sustainable disease management strategies.

Keywords: *Z. tritici*, *P. nodorum*, durum wheat, resistance, population genetics

Session 2
Oral 15

Spatio-temporal diversity of thermal responses in populations of the wheat pathogen *Zymoseptoria tritici*

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Among the environmental factors affecting the development of plant pathogens, temperature is of critical importance as it has impacts on all stages of their infectious cycle. Pathogen populations are constantly exposed to thermal fluctuations at various spatio-temporal scales. To better understand how they can respond to these changes, their adaptive potential should be taken into account. This challenge is becoming increasingly essential with the development of agro-ecology in the context of climate change, which will impact the thermal environment of foliar pathogens. The objective of this study was to assess the diversity of thermal responses both between and within natural populations of the wheat pathogen *Zymoseptoria tritici*. For this purpose, nine populations of 30 isolates each were collected by considering both: (*i*) a temporal scale with two populations sampled in the same field at the beginning and at the end of an annual epidemic (seasonal effect) ; (*ii*) a spatial scale with seven populations collected along two thermal gradients at the European continental scale (climatic zone effect). The response to temperature of each of these isolates was characterized *in vitro* by monitoring their multiplication rate in liquid culture media over four days and under 12 temperatures ranging from 6 to 34°C, then *in silico* by fitting a thermal performance curve for each isolate. Key parameters were analyzed to compare the intra- and inter-population diversity of thermal responses. Thus, this study contributes to better apprehend the diversity of responses and the existence of local adaptation patterns to temperature of *Z. tritici* at a population level. These findings will be used to infer the potential response of given populations to changes in thermal conditions and could be taken into consideration to improve prediction model of septoria tritici blotch epidemics.

Keywords : temperature, thermal performance curve, adaptive potential, spatio-temporal gradients, *Zymoseptoria tritici* populations

Session 2
Oral 16

Population biology of *Puccinia coronata* –implications for the epidemiology of crown rust on oats

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The fungus *Puccinia coronata* is the causal agent of the crown rust on oats (*Avena sativa*) and grasses. The disease is a major problem in oat growing regions and it may cause devastating yield losses. We studied the population biology of *P. coronata* in oat fields and on the alternate host in different regions to get a deeper understanding of the importance of the alternate hosts in the epidemiology of the disease. Samples were collected during a seven-year period, and collections were made from both oats and the alternate host buckthorn (*Rhamnus catharitcia*).

To explore if the role of the alternate host, on which the sexual cycle of the life cycle is completed, samples were collected in multiple years at the same farms. In addition, samples both from the alternate host, *R. catharitcia*, and oats in adjacent fields to the alternate host were collected in one year within a specified region.

We found that the genotypic variation of *P. coronata* within oat fields is high, as well as between oat fields within and between regions. The samples collected on the alternate host did not belong to the same population as the samples collected in the adjacent fields; suggesting that the presence of the alternate host greatly increases the overall genetic diversity of the fungus, however, only a few genotypes originating from *R. cathartica* will successfully infect oats. The lack of genetic structure within and between samples collected from different fields in the different regions, suggests that wind-spread spores from other *R. cathartica* hosts within a region will contribute to the genetic diversity within oat fields. This implies that the alternate host must be eradicated within a larger area to decrease the genetic diversity within *P. coronata* and limit the spread of inoculum to susceptible oat fields.

Keywords: *P. coronata* f.sp. *avenae*, population biology, epidemiology

Session 2
Oral 17

Assessment of Verticillium flax inoculum in agroecosystem soils using real-time PCR assay: from diagnosis to evaluation of crop system.

Mélanie Bressan, Manon Peyré, Adrien Blum, Lisa Castel, Jérome Ailhas, Isabelle Trinsoutrot-Gattin, Karine Laval, Christophe Gangneux.

Verticillium wilt, due to the soilborne fungus *Verticillium dahliae*, is a persistent disease affecting flax crops in Normandy. This pathology has increased since the last decade, leading to yield losses for flax producers. In part due to the long survival of *V. dahliae* in soil and the difficulty of early diagnosis, *Verticillium* flax wilt management remains problematic. Pathogen avoidance and the reduction of soil inoculum through adapted cultural practices are the two best alternatives to fight against *Verticillium* wilt.

Here, the objectives were to develop a rapid and specific assay to measure *V. dahliae* density in soil. A real-time PCR assay was optimized and validated to provide a sensitive and reliable quantification of *V. dahliae* in a range of artificially inoculated soils with known inoculum density. This assay was then successfully applied to study the *in situ* relationship between pathogen density and disease development in flax fields. Some studies have already reported the direct relationship between *Verticillium* densities in soil and disease severity on several important crops (potato, cauliflower …). But this link appeared to be more complex for flax. The importance of soil receptivity, linked to chemical and microbial characteristics, have to be particularly consider.

Finally, the real-time PCR assay was also used to evaluate and compare pathogen load in fields from diverse management systems: conventional, integrated and organic. *Verticillium* densities appeared to be impacted by agricultural practices, and particularly tillage. The influence of the previous crop on pathogen load had also to be considered with attention to manage efficiently this disease through crop rotation. Measured V. dahliae densities in all fields presented an intra-parcel heterogeneity, emphasizing the importance of an adapted sampling strategy to assess pathogen density. Such knowledge of pathogen density in soils could provide critical information for stakeholders to identify infested fields and predict disease development.

Keywords: Real-time PCR assay, *Verticillium dahliae*, Flax, soil, crop fields

Session 2
Oral 18

Incidence of root pathogens associated to clover root rot in Sweden

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Red clover (*Trifolium pratense*) is an important forage legume, however, the persistence in Swedish leys is limited by root rot associated with several fungal pathogens. For identification of soil-borne pathogens causing disease symptoms in roots fast and specific diagnostic methods were developed. The objectives were to identify the complex of soil-borne pathogens associated to root rot and to monitor disease development by qPCR in a set of red clover cultivars in field-infected red clover roots.

A field experiment was set up with eleven future and market cultivars. Pathogen identification and quantification by real-time PCR was performed in roots of three selected cultivars sampled at different time points the seeding year and once during the first and second harvest year. DNA from *Fusarium avenaceum, Phoma* spp. and *Cylindrocarpon destructans* were identified on all sampling occasions, while *F. culmorum* occurred sporadically. Significant differences in the amount of detected pathogen DNA were found within the first sampling year and between years for each of the pathogens, while no difference was shown between cultivars.

We have demonstrated that qPCR provides a useful tool for evaluating the susceptibility of red clover cultivars monitoring the pathogens causing disease symptoms, and constitutes a tool for the first step towards a disease risk assessment of clover root rot.

Round table 1

Oral 19

The use of TaqMan PCR and Next Generation Sequence Technolgy to evaluate the presence of plant pathogens in tomato seeds : What's in a Pipeline ?

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Objectives:

Evaluate NGS technology for the use of detection of plant pathogens in tomato seeds compared to real-time TaqMan PCR

Methods used:

DNA and RNA extraction from spiked and unspiked tomato seeds, TaqMan PCR, Illumina HiSeq sequence, pipeline analysis of sequence data

Results:

Seeds are transported all over the world. Many different pathogens of tomato can be transmitted by seed and therefore monitoring seed health is essential to reduce contamination risks. For the detection of these seed transmitted pathogens many methods are available, e.g. ELISA, IF, dilution plating, PCR and real-time PCR. We evaluated TaqMan PCR methods of tomato seed transmitted pathogens and the use of Next Generation Sequence Technology with a focus on: *Clavibacter michiganensis* subsp. m*ichiganensis*, *Xanthomonas euvesicatoria, X. perforans*, *X. vesicatoria* and *X. gardneri, Pseudomonas syringae* pv. *tomato*, Pepino Mosaic Virus and Potato Spindle Tuber Viroid. The TaqMan PCR assays were shown to be species specific when tested on a series of pathogens and related species. Different DNA and RNA extraction kits were evaluated in combination with the KingFisher platform. Validation of the complete method was performed for the different performance characteristics: analytical sensitivity, analytical specificity, repeatability, reproducibility, selectivity and robustness using seed bags containing healthy seed lots spiked with the different tomato pathogens. Obtained DNA- and RNA extracts were evaluated with Illumina HiSeq analysis for the pathogens included in the study.

Conclusions:

An efficient DNA/RNA extraction procedure was developed for tomato seeds. TaqMan PCRs were validated on a set of tomato seed transmitted pathogens. These DNA extracts were also used for Illumina HiSeq analysis. We found a good correlation between results obtained by the TaqMan assays and the NGS analysis to demonstrate the presence of the pathogens .

6 key words:

DNA/RNA extraction, tomato seeds, TaqMan PCR, NGS

Round table 1
Oral 20

The application of next-generation sequencing (NGS) offers great opportunities for the detection and diagnosis of viruses and viroids in plants

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The current widely used molecular methods for detection and identification of viruses and viroids in plants, mostly RT-(q)PCR and Sanger sequencing, have their limitations. First of all, the need to make the right choice of the virus-specific molecular test has an important impact on the reliability of the test result. Secondly, in case of multiple infections, the correct conclusion is not always drawn, and thirdly, unexpected presence of viruses or viroids may easily remain unresolved and related symptoms may lead to the false conclusion of a "non parasitic cause". Next generation sequencing (NGS) or deep sequencing follows a massively parallel sequencing (MPS) approach, a powerful technology offering a huge potential for holistic virus/viroid identification in all kinds of (suspected) plant tissues and this without preceding knowledge on the target sequences. Within the framework of the transnational EUPHRESCO project "NGS Detect", a group of 15 partners is working towards developing and adapting a standardized NGS technology for viruses and viroids in plants. In a first series of experiments, ILVO has assessed several key aspects, starting from the subsampling procedure, up to the comparison of data analysis strategies, following NGS on an Illumina platform. Several host plants were used (e.g. apple, pear, potato, cherry), both with known and unknown virus infections. Initially, partial virus enrichment (purification by ultra centrifugation) was compared with a direct RNA extraction on the plant tissue. However, the virus purification procedure did not lead to sufficient RNA of yield and integrity to pass the quality control (QC) and was abandoned. Further, the effect of three total RNA extraction methods (Trizol and 2 kit extraction methods, RNeasy (Qiagen) and mirVana (Ambion)) on the NGS results were mutually compared. The results of these methods based on total RNA extraction were also compared to a protocol starting from small RNAs extracted directly from the plant tissue (mirVana (Ambion) siRNA extraction). In addition, the effect of rRNA depletion was evaluated. Library preparation and NGS were outsourced (Admera Health, USA). Finally, the data were analysed using in-house scripts and through an automated pipeline for virus detection in the NGS data (VirusDetect pipeline; Zheng et al. 2017). Currently, a comparison with a second automated pipeline (Virtool; ©2016 Canadian Food Inspection Agency) is being evaluated, both for the detection of known, and "new" viruses. All obtained NGS results were confirmed and evaluated by specific detection with the available classic molecular tools. The current evolution in continuous cost reduction for the routine use of NGS also lets presume that in the very near future, once an optimised strategy will be available, the technology may be widely implemented by plant virus research and diagnostic labs.

Keywords: next generation sequencing, NGS, deep sequencing, bioinformatics, siRNA

Round Table 1
Oral 21

Rapid and accurate identification of *Ralstonia solanacearum* "species complex" by MALDI-TOF MS.

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Ralstonia solanacearum "species complex" (RSSC) is a soil-borne plant pathogenic bacterium, consisting of diverse and widespread strains that cause bacterial wilt on a wide range of host plants, among them many ornamental plants. Recently, we reported a unique finding of RSSC, Phylotype I, resulting in stunted, yellowing and wilted ornamental *Rosa* sp. plants cultivated under greenhouse conditions. Identification of RSSC isolates from *Rosa* sp. was, among other tests, performed at the proteomic level using Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS). Prior to the routine identification of RSSC isolates from ornamental *Rosa* sp. and associated water samples, we created spectral database entries (Mass Spectra Profiles, MSP's) specific for the RSSC, that represented all the four phylotypes. All spectra were obtained in linear positive-ion mode with an m/z range of 2000–20,000 Da. Validation data demonstrated that MSP's created were specific for the identification of the representatives inside the RSSC, clearly discriminating them from other related *Ralstonia* sp., such as the *R. picketii*. Additionally, the protein profiles we acquired in the present study accurately identified RSSC at the species level, including *R. pseudosolanacearum* (phylotypes I and III), the "real" *R. solanacearum* (phylotype II) and *R. syzygii* (phylotype IV), describing the diversity of the *R. solanacearum* "species complex".
For routine use, the MALDI-TOF MS was additionally shown to be robust, quick and reproducible.

Key words: MALDI-TOF, RSSC, phylotypes, *Ralstonia solanacearum, Ralstonia pseudosolanacearum*, *Rosa* sp.

Round table 1
Oral 22

Comparison of Multispectral imaging and Near infrared Reflectance Spectroscopy methods for phenotyping resistance of cereals to Fusarium Head Blight (FHB) and Deoxynivalenol (DON)

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To encourage development of cereal varieties resistant to Fusarium Head Blight and mycotoxins for the National List, two new methods of phenotyping were developed by GEVES on winterwheat. They are more reliable and faster than in-field visual assessment and cheaper than LCMS-MS for measuring DON content. Multispectral imaging is being developed to predict /quantify damage from diseases on several species. Near-Infrared Spectroscopy is an efficient and non-destructive method for seed quality analysis. The aim was to assess the potential of these technologies for quantifying *Fusarium graminearum* damage, compared to visual assessment and DON content in kernels. i) With Multispectral imaging (Videometer^{R}), the algorithm "Fusa-spectrale wheat", was developed to evaluate the percentage of Fusarium Damaged Kernels (FDK) at maturity for varietal classification using Canonical Discriminant Analysis between kernels infected/not infected, on three years at several sites. FDK is strongly correlated with percentage of scabbed spikelets assessed by visual scorings (R>0.87) and with DON content (R>0.88). Correlation between FDK and *Fusarium graminearum* biomass assessed by real-time PCR was stronger in trials with only *F. graminearum* (R=0.97) than trials with a complex *Fusarium-Microdochium.* For durum wheat and triticale, studies are ongoing. ii) NIR calibration was developed from a sample set of varieties produced in two locations over two crop years. Calibration models (visual scoring, DON content) were developed using Partial Least Square Regression (PLS). Each model was checked by internal cross validation. Their performance was assessed by the coefficient of determination in calibration (R^2cal) and standard error in cross validation. The R^2cal observed was 0.90 for "DON content" model and 0.79 for "visual scoring" model.
These two technologies show potential to evaluate FHB and DON resistance on cereals. The NIRS models require additional data to include geographical, genotyping and years effects. With multipectral and hyperspectral imaging, research is ongoing to quantify *Microdochium* spp.

Key words: *Fusarium graminearum,* Phenotyping, Deoxynivalenol, Near-infrared spectroscopy, Multispectral imaging, Cereals.

Round Table 1
Oral 23

Stable insertion and transient expression of green fluorescent protein in *Aphanomyces euteiches,* the causal agent of pea root rot disease

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Pea (*Pisum sativum*) root rot disease is caused by the oomycete pathogen *Aphanomyces euteiches. A. euteiches* is one of the most destructive soilborne pathogens of pea crop. Since its discovery, no efficient control methods have been reported. Currently, the details of *A. euteiches* infection mechanisms are still not well known. In order to study the interaction between *A. euteiches* and pea root, an *A. euteiches* strain expressing the reporter gene *gfp* (Green Fluorescent Protein) has been obtained. A large quantity of protoplasts from a young *A. euteiches* mycelium was produced using lysing enzymes from *Trichoderma harzianum* and 10% could regenerate on PDA medium supplemented with Mannitol. The protoplasts were transfected by PEG/CaCl2-mediated method with *Aequorea victoria gfp* gene under the control of a strong constitutive promoter from the *Bremia lactucae Ham34* gene and fused to the terminator *Ham34* gene. The data show for the first time that transient expression of *gfp gene* can be obtained in *A. euteiches* mycelium. This result confirms that a promoter from a Peronosporale oomycete can be expressed in a Saprolegniale oomycete. qPCR analysis and confocal microscopy observation confirmed both insertion and expression of *gfp* in *A. euteiches* mycelium. However, despite the multiple insertion of the *gfp* gene in the *A. euteiches* genome, only a transient cytoplasmic expression of GFP could be observed. The loss of the GFP fluorescent signal may result from a gene silencing process as previously demonstrated for the *Phytophthora infestans* elicitin *inf1* gene (van West et al., 1999).

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Keywords: *Aphanomyces euteiches*, oomycetes, soilborne pathogen, transfection, GFP

Acknowledgments

This research is supported by the Regional Federation for Research “Nomandie-Vegetal”. GRR-VASI is acknowledged for financial support.

Session 3. EMERGING DISEASES AND NEW INSIGHTS IN TAXONOMY AND PHYLOGENY OF PLANT PATHOGENS IN EVOLVING GLOBAL CONDITIONS

Keynote lecture 5

Keeping up with the plant destroyers in the post-genomics era

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Infectious plant diseases cause havoc to world agriculture and threaten to slow laudable efforts to launch a second green revolution to meet the food security needs of a booming world population. Pathogens such as the rice blast fungus, wheat stripe and stem rust, the Irish potato famine pathogen, and many others continue to trigger recurrent epidemics with far reaching consequences. When faced with opponents like these, we need to know our adversary. The genome sequence of a plant pathogen is a deep look into its soul. From important and often unexpected insights into the biology of the pathogen to the tools needed to develop surveillance and diagnostic DNA markers, the genome is an invaluable resource that accelerates research and output delivery. With the cost of genome sequencing decreasing even faster than Moore's law, the cost-benefit calculation is evident. For instance, countless time and money are spent in developing DNA markers, investigating population structures, debating the pathogen origin, etc. – activities that can be greatly hastened by the genome sequence. In this talk, I will discuss some of ways in which genome biology impacts plant pathology. In particular, I will address how pathogen genomics can drive basic and applied plant pathology, and how state of the art findings on pathogen biology can be exploited to drive the development of new approaches to breeding disease resistant crops. Detailed knowledge of the pathogen genome coupled with novel methods of plant genome editing is ushering the era of next-generation disease resistance breeding in plants.

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Session 3
Oral 26

Genomic study shows endemic and pandemic rice blast lineages and lineages between rice and setaria infecting gene pools

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The rice blast fungus *Magnaporthe oryzae* is the most damaging rice pathogen, and a textbook example of widely distributed, rapidly adapting pathogen, despite limited genetic diversity. The aim of our study was to elucidate the factors and evolutionary changes underlying the emergence, diversification and spread of *M. oryzae* on rice. Analyses of population structure based on Infinium-genotyping of 5300 SNPs for 970 isolates collected on rice on the five continents identified 13 lineages within *M. oryzae*. Three lineages were pandemic in multiple continents and one of them was the only lineage detected in Europe. Multiple lineages with more restricted distributions in sub-Saharan Africa and Asia were also identified. Whole genome resequencing of a subset of 80 rice-infecting isolates combined with 12 isolates collected on *Setaria* millet revealed several lineages with intermediate positions between the previously identified rice- and *Setaria*-infecting gene pools, questioning the *Setaria* origin of rice-infecting *M. oryzae* and suggesting that the emergence of rice blast may be more recent than previously thought. Because the sequenced isolates were collected between 1973 and 2009 and recombination is limited, we will use dated tips to calibrate tree nodes within a phylogenetic framework to elucidate the timing of emergence and global dispersal of *M. oryzae*. Most lineages were highly clonal, but we found evidence for recombination in a widely distributed lineage infecting upland rice in Yunnan, Laos and Thailand. We will use genome scans for genetic exchanges to test the hypothesis that recombining lineages are more likely to receive genetic material from other lineages. Our work provides a population-level genomic framework for defining molecular markers to assist in the control of rice blast and for investigating the molecular underpinnings of phenotypic and fitness differences between divergent lineages.

Keywords : *Magnaporthe*, *Setaria*, Rice, Population genomics, Recombination, Gene flow

Session 3
Oral 27

Assessing the phenotypic and genotypic diversity of *Sclerotinia sclerotiorum* in France

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Sclerotinia sclerotiorum is a highly polyphagous ascomycete that can attack more than 400 plant species among which vegetables and arable crops. In order to improve the efficiency of plant protection against white mould, knowledge on the *S. sclerotiorum* populations has to be acquired. In the framework of national project "Scleroleg", *S. sclerotiorum* isolates were collected on symptomatic plants in several French regions. Their genetic and phenotypic diversity was assessed.

The aggressiveness of 103 isolates collected from bean, canola, carrot, lettuce, melon and witloof chicory was compared on melon, tomato and lettuce in controlled conditions. All isolates were able to cause symptoms on detached leaves of the three plant species. The relative level of aggressiveness of an isolate, compared to the others, tended to be similar regardless of the plant species on which it was assessed. On average, the isolates collected from lettuce were slightly more aggressive than the others on all three host plants tested. For the rest of the isolates, there was no link between host of origin and aggressiveness, suggesting that there is no marked host specialization of the fungus. These data were partly supported by the results of genetic characterization of 200 *S. sclerotiorum* isolates with 16 microsatellites markers. While there was no significant geographic differentiation among isolates (some haplotypes were shared by isolates collected on different species and separated by up to 600 km), the lettuce isolates showed strong and significant genetic differentiation with all others.

The reasons for these differences remain to be elucidated. However, our study clearly suggests that sclerotia of *S. sclerotiorum* generated on one host crop will likely be capable of providing inoculum for the next susceptible crop grown in the same plot and possibly for crops in neighbouring fields, via the production of airborne ascospores.

Keywords: white mould, epidemiology, aggressiveness, genetic differentiation

Session 3
Oral 28

Modelling the dispersal of *Monochamus galloprovincialis,* the vector of the pine wood nematode, and assessing the effectiveness of clear-cutting measures

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Following the detection of a pine wood nematode outbreak, Implementing Decision 2012/535/EU imposes eradication measures for at least four years, mainly consisting of the establishment of: i) clear-cuts with a radius of 500 m around the infested plants and ii) intensive surveillance within a radius varying from 6 to 20 km around the infested zone. To estimate the effectiveness of these measures, a simulation model was developed and calibrated using experimental data specifying the dispersal capabilities of the insect vector *Monochamus galloprovincialis.* These data were obtained in the laboratory (on a flight mill) and *in natura* (mark-and-recapture experiments) in the context of the Landes de Gascogne forest (David, 2014 and David *et al.*, 2014), one of the forest areas most exposed to the risk of introduction of the pine wood nematode in France. The simulations that were then carried out under different scenarios (preventive or curative) showed that the clear-cuts currently requested by European and French regulations would not be effective in a landscape configuration of continuous plantations of maritime pine. Indeed, with the recommended radius of 500 m, at best 11% of nematode transmissions by the insect vector would be avoided. Moreover, to obtain a pine wood nematode transmission rate lower than 0.1%, it would be necessary to implement clear-cuts with a radius of 15 - 38 km (Anses, 2015). These simulations, carried out in the context of a continuous forest, should however be supplemented by a scenario involving highly fragmented pine forests. In this regard, it would be interesting to include biological data from Spanish forest managers, as they are directly involved in implementing nematode eradication measures in this type of landscape. Alternative management measures to control the spread of the pine wood nematode were also assessed by the Working Group.

Keywords: Pinewood nematode, *Monochamus galoprovinicalis,* Dispersion, Clear cuts, Risk assessment

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Session 3
Keynote lecture 6

Risk assessment and mitigation of the introduction and spread of new plant pathogens in a changing world

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An overview will be given of major global trends, including increasingly uneven resource distribution, increased war and migration, globalization of food production, trade and consumption, increasing inequalities and food insecurity, environmental degradation, global climate change, emerging pathogens, and increasing ineffectiveness of fungicides and antibiotics. Then I will zoom in on emerging plant pathogens associated with some of these global changes. I will discuss current tools for risk assessment of the potential introduction and spread of new plant pathogens, with a focus on correlative prediction models combined with GIS and microbial risk assessment models and the associated uncertainties. I will use Banana Xanthomonas wilt, Citrus Huanglongbing and Blueberry twig blight as examples. Mitigation options will focus on quality and sustainability certification, quarantine measures, early detection using internet-based electronic tools, removal of infected plants (when and where appropriate), development and use of plant resistance, and prevention of spread by enhancing agroecosystem resilience by increased plant and microbial diversity. The presentation will end with calling on plant pathologists to contribute to solving global problems, in particular environmental degradation and food insecurity.

Session 3
Oral 29

BiOR² : a database/software process dedicated to plant pests and pathogens ranking
Session 6: Emerging tools for the management of plant diseases: agroecology and disease management

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In the context of globalization of plants trade, models for ranking invasive pests according to the risks for crops and environment are important to risk managers as they have to adapt quickly management strategies according to risky pathways. To this end, a new methodology was developed: BiOR², which stands for "Biological Organisms data Retrieval and Ranking system".
BiOR² combines a database to graphic interfaces and multi-criteria decision aiding (MCDA) method. First, the database runs under the database management system (DBMS) PostgreSQL. It consists of 55 tables containing information relative to plants' trade, land use, legislation, pest interceptions and climate classification. The database is linked to a graphic user interface (BiOR² Form) that allows filling in data on host plants, pathogens and pest-plant interactions through a questionnaire. Next, another graphic user interface (BiOR² Statistics) constructs a multi-criteria matrix by relying on 2800 lines of codes, enable the link between the available data according to specific criteria and whose uncertainty can be also quantified by BiOR². A base of 24 independent criteria, defined according to FAO ISPM N°11 document, covers risk of entry, establishment, spread as well as economic, social and environmental impacts. The ranking is finally achieved through Visual PROMETHEE (MCDA method) with the possibility of conducting several scenarios according to the occurrence of the pest in the area of interest or through an adaptable criteria weighting system.
The process was applied to metropolitan France and the French Oversea Departments on respectively 278 and 110 plant pests and pathogens, which demonstrates its applicability to several regions. The contribution of BiOR² to policy recommendation such as pest prioritization was also investigated. Overall, BiOR² is intended to be objective, generic, based on sound science, highly adaptable to meet various kinds of questions about the management and impact of pests and pathogens.

Keywords: Plant pests and pathogens, plant health, ranking, risk assessment, database, multi-criteria analysis

Session 3
Oral 30

Large host jumps in some powdery mildew species revealed by phylogeny and cross inoculations

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Powdery mildew fungi have long been considered as highly specific pathogens, i.e. a given species can infect only a narrow range of host plants, usually in the same genus. The availability of molecular markers has dramatically improved and changed our vision on these fungi, especially that host specificity is not as simple and narrow as previously believed. For example, recent phylogenies have strongly suggested that species causing oak and mango powdery mildew are closely related. Here we analysed mango powdery mildew samples from southern Spain (6 different orchards in Malaga region) by combining multi-gene phylogeny (ITS + 4 single-copy coding genes) and cross inoculations between oak and mango. Based on genetic analyses, two *Erysiphe* species were identified in mango samples, which are the species usually associated with oak powdery mildew in Europe. *Erysiphe quercicola* was dominant with 97% positive samples, whereas *E. alphitoides* was found in only 11% of all samples, 3% alone and 8% in mixture with *E. quercicola*. Cross inoculations between oak and mango, which led to typical symptoms, further supported the conspecificity of oak and mango powdery mildew species. These results constitute the first report of mango powdery mildew in mainland Spain and mainland Europe, caused by *E. quercicola* and *E. alphitoides*. Our study confirmed the broad host range and/or host shifting ability of both *E. quercicola* and *E. alphitoides*, which may explain their invasive success. Furthermore, it opens interesting prospects to the elucidation of molecular mechanisms underlying host range since the two closely related *Erysiphe* species belong to a small clade with both generalist and specialist powdery mildews.

Keywords: powdery mildew, host range, host shift, biological invasion

Session 3
Oral 31

Identification and characterization of *Phytophthora* hybrids using genotyping-by-sequencing

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Since the mid-1990's, several *Phytophthora* hybrids have been described in multiple phylogenetic clades. As a result of intensified international plant trade, which might bring previously separated species in contact with each other, an increase in the number of hybridization events is suspected. These hybrids might be more aggressive or have an expanded host range in comparison to the parental species. Early detection of new hybrid species is thus warranted, but is not always successful via traditional PCR amplicon sequencing of individual loci. We have used two-enzyme genotyping-by-sequencing (GBS) to reliably identify and characterize hybrids in a European collection of several hundreds of *Phytophthora* isolates.
DNA was extracted, digested and GBS-adapters were ligated. After PCR amplification, GBS libraries were quantified, pooled and sequenced using the Illumina HiSeq 3000 technology. Read data was preprocessed using custom scripts and subsequently subjected to the GibPSs pipeline to identify a global set of GBS tags across all samples. Subsequently, per sample the absence/presence profiles were scored together with SNP profiles for each GBS tag. The method was highly reproducible and generated about 15000 to 30000 tags for a non-hybrid species. When isolates share a large number of GBS tags with at least two *Phytophthora* species, this is indicative of a hybridization event. Using this approach, we detected a previously unidentified hybrid of *P. chlamydospora* and *P. lacustris*. Even when the parental species are not known, a large number of GBS tags in a sample can point to a hybridization event, as hybrids contain tags from each of the parental species. We thus identified a number of potential novel hybrid species, which implies that historic hybridization events might be more common in *Phytophthora* than previously assumed. Genotyping-by-sequencing can thus be used to reliably identify and characterize *Phytophthora* hybrids

Session 4. MICROBIAL DETERMINANTS OF PATHOGEN AND SYMBIOTIC INTERACTIONS

Keynote lecture 7

Understanding pathogen adaptation to the plant host

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Filamentous plant pathogens pose a severe threat to global food security. These organisms often show exquisite host adaptation, but also undergo rapid evolution leading to shifts or expansions in the host range. The genetic mechanisms of pathogen-host adaptation remain poorly understood. In the soil-inhabiting vascular wilt fungus *Fusarium oxysporum,* individual isolates tend to exhibit high specificity towards a given plant host, while the species complex collectively attacks more than a hundred different crops. In addition, *F. oxysporum* is also an emerging human pathogen that provokes lethal systemic infections in immunocompromised individuals. Remarkably, a single field isolate of this fungus can kill tomato plants, immunodepressed mice and insects. By following a combination of reverse genetics and experimental evolution approaches, we found that *F. oxysporum* uses multiple strategies to adapt to different host environments. These include recruitment of conserved fungal signaling pathways or hijacking of host regulatory mechanisms for new virulence-related functions. Strikingly, fungal populations evolved after serial passaging through different environments displayed large-scale chromosomal reorganizations in transposon-rich accessory regions of the genome, suggesting that chromosome plasticity could act as a major evolutionary driver in *F. oxysporum*. Understanding the genetic mechanisms that govern virulence evolution and host adaptation may reveal new ways to control diseases caused by filamentous pathogens and improve plant health.

Keywords: evolution, fungus, *Fusarium*, host, pathogen, virulence

Session 4
Oral 32

Identification of atypical chitin synthase genes horizontally transferred in microbial plant pathogens

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Chitin, the second most abundant biopolymer on earth after cellulose, is found in probably all fungi, many animals (mainly invertebrates), several protists and a few algae, playing an essential role in the development of many of them. It is produced by type 2 glycosyltransferases, called chitin synthases (CHS). In phytopathogenic fungi, several CHS isoenzymes are found and they are not only thought to be essential for their growth but they are also considered to participate as determinants of their pathogenicity .

We performed a genome-wide analysis of the CHS multigenic family and created a databank of putative CHS. Phylogenetic analyses first allowed to propose a robust and unifying fungal CHS classification that is easily accessible through a dedicated website (http://wwwabi.snv.jussieu.fr/public/CHSdb). These analyses also permitted to study the evolutionary history of CHS. This family has mainly evolved via duplications and losses. However, it is likely that several horizontal gene transfers (HGT) also occurred in eukaryotic microorganisms and, even more surprisingly, in the genomes of bacteria. Moreover, some fungal CHS are highly similar to CHS of giant algae-infecting viruses. Interestingly, many of these atypical CHS are found in plant pathogens, bacteria or fungi. Different characteristics of these CHS genes might be associated to virulence factors implied in plant interactions.

Keywords: Chitin synthase, Evolution, Bacteria, Viruses, Fungi, Horizontal gene transfer.

Gonçalves *et al.* BMC Evolutionary Biology (2016) 16:252.

Session 4
Oral 33

Colonization of plants by pectobacteria: more than just a "brute force"

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Pectobacteria are considered as "tough" phytopathogens that colonize their hosts applying "brute force" – extracellular enzymes damaging plant tissues. However, some observations point to the significance of pectobacteria "stealth" behavior *in planta*. An important property of microbial populations is related to their heterogeneity. Herewith, different morho-physiological cell types are functional parts of the population. The research of the formation of differentiated microbial cells is in its infancy, especially within host/pathogen systems.

We tested if the colonization of plants is coupled with dissociation of pectobacteria population and tried to obtain information on causes and consequences of functional specialization of pectobacteria cells *in planta*.

We have "looked inside" infected plants using a variety of complementary methods: various types of microscopy and chromatography, gene cloning and their heterologous expression, mutagenesis, gene expression analyses (qPCR, RNA-Seq).

Our results show that different plant compartments form specific signaling backgrounds that drive the behavior of microbes resulting in *in planta* dissociation of bacterial population. Herewith, various sub-populations display functional specialization (blocking the vessels, stress adaptation, tissue maceration, cell migration, etc.). Novel biofilm-like structures – bacterial emboli were described and discussed to play significant roles in the formation of pathosystem. *In planta* dissociation of bacterial population is coupled with plant susceptible response that provides conditioning of the colonized compartment. Our observations are strengthened and deepened by the whole-transcriptome analysis of both plant and pathogen during their interactions. This study was supported by RSF (15-14-10022).

Keywords: Soft rot, dissociation of population, biofilms, bacterial emboli, plant susceptible response

Session 4
Oral 34

Fungal effectors and plant regulators of the barley susceptibility factor RACB

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The grass powdery mildew fungus *Blumeria graminis*, as a haustorium-forming ectoparasite, has to overcome pre- and post-invasive host immunity. Additionally, as an obligate biotroph, *B. graminis* has to reprogram the host for changes in host cell architecture and physiology, but little is known about virulence effector functions that support this.

The signalling G-protein RACB is a barley susceptibility factor and a regulator of host cytoskeleton dynamics. Activated RACB supports fungal invasion into epidermal cells of barley and haustorium expansion. RACB´s function as a susceptibility factor is tightly controlled in the host. MICROTUBULE-ASSOCIATED ROP GTPASE ACTIVATING PROTEIN1 controls RACB at the level of signalling activity whereas cytoplasmic ROP-BINDING KINASE1 controls RACB protein abundance. However, the physiological function of RACB is not fully understood.

Recent results show that RACB does not function as a negative regulator of PAMP-triggered immune responses in barley. Instead, RACB is required for polar development of barley epidermal cells. Strikingly, we found that *B. graminis* expresses an effector we called ROP-interactive peptide 1 (ROPIP1) from a non-autonomous retroelement. ROPIP1 interacts with RACB in planta, is translocated into the host cytoplasm and destabilizes host microtubules. ROPIP1 has virulence supporting functions when over-expressed in the host, and host-induced gene silencing of ROPIP1 limits fungal penetration success.

Apparently *B. graminis* evolved a novel type of virulence effector by neo-functionalization of a repetitive element. This effector targets RACB, which otherwise functions in polar cell growth and microtubule organization. This suggests that RACB´s function is co-opted by *B. graminis* for reprogramming of host cell cytoskeleton and development during ingrowth of the haustorium.

Keywords: Blumeria graminis, barley, small GTPase ; microtubules, effector-triggered susceptibility

Session 4
Oral 35

The biological and evolutionary basis of systemic plant pathogenesis in *Xanthomonas*

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Pathogenic microbes cause systemic and non-systemic diseases of plant and animal hosts. Systemic diseases are particularly destructive because the pathogen moves through the host vasculature causing widespread infection; meanwhile non-systemic pathogens remain restricted to the non-vascular tissue near the site of infection. The basis of systemic and non-systemic pathogenesis is unclear. Here we describe the role of cell wall degradation in the evolution and biology in the Gram-negative phytobacterial genus *Xanthomonas*. *Xanthomonas* comprises a diverse group of vascular and non-vascular pathogens of over 200 plant species. We demonstrate that a single, vascular pathogen-unique cell wall degrading enzyme called CelA contributes to systemic pathogenesis in multiple pathogenic lineages in this diverse genus. We determined that CelA1 was conserved only in systemic pathogenic bacteria in the genera *Xanthomonas, Xylella* and *Ralstonia* but absent in non-systemic Gram-negative plant pathogenic bacteria. Most notably addition of this cell wall degrading enzyme to two distinct non-systemic pathogen species, barley-infecting *Xanthomonas translucens* and rice-infecting *Xanthomonas oryzae*, permitted systemic pathogenesis of their respective host plants. Further genomic analysis of non-systemic *Xanthomonas* pathogens appear to have inactivated this trait suggesting that they arose from related vascular subgroups upon adapting to the non-vascular plant environment. Overall this work provides a framework to describe pathogen emergence based on symptom development and tissue-specificity in an important pathogen genus.

Keywords: Evolution, *Xanthomonas*, Systemic pathogenesis

Session 4
Oral 36

A new proteinaceous PAMP from Ascomycetes induces cell death in *Solanaceae*

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Recognition of conserved pathogen- or microbe-associated molecular patterns (P/MAMPs) by plants initiates PAMP-triggered immunity (PTI). Some PAMPs elicit defence responses in a wide range of plant species, while response to others is restricted to certain plant species, probably reflecting the presence of a particular cell surface localized pattern recognition receptor (PRR). Although a number of microbial PAMPs have been identified the full repertoire remains unknown.

The causal agent of scald, *Rhynchosporium commune*, is one of the most destructive and economically important pathogens of barley. It is a hemibiotroph with an extended asymptomatic phase. Following conidia germination and cuticle penetration *R. commune* hyphae spread between barley epidermal cells without directly penetrating them.

Sequencing of the *R. commune* transcriptome from an early time point during barley colonisation revealed a highly abundant transcript encoding a small secreted fungal protein of unknown function with four cysteine residues, which we called RcCDI1 (Cell Death Inducing). It is most highly abundant at the onset of barley infection with *R. commune*. *Pichia pastoris* was used to produce RcCDI1 and its homologues from different fungal species, including *Zymoseptoria tritici*, *Magnaporthe oryzae* and *Neurospora crassa*. All of these proteins exhibited PAMP activity inducing cell death in *Solanaceae* but not in other families of dicots or monocots. Virus-induced gene silencing (VIGS) of known components of PTI in *Nicotiana benthamiana* showed RcCDI1-triggered cell death to be BAK1, SOBIR1 and SGT1 dependent. However, the cell death was not suppressed by the *Phytophthora infestans* effectors PiAVR3a or PexRD2, suggesting that it does not require NbCMPG1 or NbMAPKKKe. Identification of the plant receptor involved in RcCDI1 recognition in *N. benthamiana* will provide a valuable resource for engineering non-host resistance in monocots.

Keywords: cell death, fungal, PAMP, pathogen, *Rhynchosporium commune*, Ascomycete

Session 4
Oral 37

Unusual evolutionary mechanisms to escape Effector-Triggered-Immunity in the fungal phytopathogen *Leptosphaeria maculans*

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Leptosphaeria maculans is the fungus responsible for the stem canker disease of oilseed rape (*Brassica napus*). *AvrLm3* and *AvrLm4-7*, two avirulence genes of *L. maculans*, are involved in an unusual relationship: *AvrLm4-7* suppresses the *Rlm3*-mediated resistance. Surveys of populations submitted to the *Rlm7* selection pressure recently showed that the loss of the *AvrLm7* specificity was accompanied by the gain of avirulence toward *Rlm3*.

In order to better understand the molecular bases of this unusual relationship, and following the cloning of *AvrLm7* and *AvrLm3*, we assessed the molecular polymorphism of *AvrLm3* in a world-wide collection of 235 *L. maculans* isolates. No field isolates exhibited deletion or inactivating mutations in *AvrLm3*, as observed for other *L. maculans* avirulence genes. A high level of nucleotidic polymorphism was also found, and eleven isoforms of the AvrLm3 protein were found. Signatures of positive selection were identified in *AvrLm3.*

In isolates virulent towards both *Rlm3* and *Rlm7* (a3a7), the loss of the *Rlm3*-mediated response was due to two distinct mechanisms. When *AvrLm4-7* was inactivated (deletion or inactivating mutations), amino acid substitutions in AvrLm3 generated virulent isoforms of the protein, responsible for virulence towards *Rlm3*. However, half of the a3a7 isolates still contained an avirulent allele of *AvrLm3*, along with point mutations in *AvrLm4-7*. Directed mutagenesis confirmed that some point mutations in *AvrLm4-7* were sufficient for the fungus to escape *Rlm7*-mediated resistance while maintaining the suppression of the AvrLm3 phenotype. The complex evolutionary mechanisms enabling *L. maculans* to escape *Rlm3*-mediated resistance while preserving *AvrLm3* integrity, along with observed reduced aggressiveness of isolates silenced for *AvrLm3,* suggest this effector plays a major role in pathogenicity towards *B. napus*. This example contributes to complexify the gene-for-gene concept of plant-pathogen evolution with a "camouflaged" model allowing retention of non-dispensable avirulence effectors.

Fundings : CTPS-ONEMA "ICOSCOP", INRA metaprogramme SMaCH (K-masstec project). ANR **(LabEx BASC; ANR-11-LABX-0034).**

Keywords: *Leptosphaeria maculans*, evolution, *Rlm7*, *Rlm3*, avirulence genes, *Brassica napus.*

Session 4
Oral 38

Clathrin, a key role in the delivery of virulence factors in the phytopathogenic fungus *Botrytis cinerea*

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Fungi are the most important plant pathogens on agricultural and horticultural crops. Study of these pathogens remains essential to understand pathogenic process and control plant diseases. These organisms evolved a repertoire of secreted enzymes involved in plant decomposition to colonize their hosts. Secretory proteins are transported from Endoplasmic Reticulum and Golgi apparatus to extracellular space through intracellular vesicles. In filamentous fungi, intracellular vesicles were observed using electron microscopy but their biogenesis process is still unknown. The elucidation of this process and the identification of proteins involved in secretory vesicles biogenesis remains a challenge to understand virulence factors delivery. A nonpathogenic mutant altered in the expression of a gene encoding clathrin heavy chain was selected in a random mutant library generated in the necrotrophic pathogen *Botrytis cinerea*. This gene is essential in many organisms. A clathrin dominant negative mutant was generated in *B. cinerea* and confirmed the nonpathogenic phenotype observed on several host plants. In eukaryotic cells, clathrin heavy chain is mainly described in the endocytic pathway, but it is also essential for high-density secretory vesicles formation in *S. cerevisiae* (Gurunathan *et al*., 2002). Characterization of the two mutants of *B. cinerea* revealed a secretion defect of numerous proteins including known virulence factors, as Plant Cell Wall Degrading Enzymes and elicitors. A clathrin-coated vesicle enriched fraction was isolated from the wild type and mutants strains. Using a proteomic approach, we analyzed and compared the proteomic composition of these clathrin-coated vesicles and present here the results.
This study demonstrated for the first time the essential role of clathrin in the infectious process of a fungal pathogen and its importance in virulence factors secretion.

Keywords: clathrin, vesicles, secretion, LC-MS analysis, virulence

Session 5. FROM PLANT IMMUNITY TO INNOVATIVE PLANT BREEDING

Keynote lecture 8

Biotechnology for Plant Disease Control - *Novel tools and perspectives*

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Faced with the multiple challenges of (1) predicted world population growth, (2) urbanisation reducing available area of agricultural land, (3) an increased demand for meat-based diets and therefore fodder and (4) climate change and catastrophic weather events, global agriculture must adopt more dynamic, efficient and sustainable production methods to increase food and fodder production in order to support a growing population (FAO).
Biotechnology platforms already play an integral role in improving agricultural yields and food quality. With the use of molecular breeding and mutational tool, farmers are producing record yields than ever previously. By providing both innovative tools in plant breeding and uncovering fundamental knowledge of plant-microbes interactions, these new approaches also pave the way forward in advanced disease management.
I will provide an insight into techniques including and beyond transgenic approaches in plant biotech applications. CRISPR-Cas9 will be highlighted as a key biotechnological but non-transgenic tool for the development of disease resistant plants. Innovations in this field stand to improve plant disease control methods for future farming systems.

Collinge DB Biotechnology for Plant Disease Control, New York and London: Wiley (2016) 440 pages. ISBN 978-1-118-86776-1 – URL for Book: http://eu.wiley.com/WileyCDA/WileyTitle/productCd-1118867769.html

Session 5
Oral 39

Uncovering the priming potential of the Green Leaf Volatile Z-3-HAC in wheat, a metabolomics approach

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Because of growing environmental concerns and following legislative pressure (2009/128/EC), the need for innovative crop protection tools has grown during the last decade. Within the context of integrated pest management (IPM), green leaf volatiles (GLVs) may be of particular interest. GLVs are ubiquitously produced throughout the whole plant kingdom and have been attributed an important role in direct and indirect defense responses and in the priming of plant defense. Priming refers to a mechanism whereby plants are sensitized to respond faster and/or more strongly to future pathogen attack.
In previous research, we demonstrated that pre-exposure to the green leaf volatile (GLV) Z-3-hexenyl acetate (Z-3-HAC) primed wheat (*Triticum aestivum* L.) for enhanced defense against subsequent infection with the hemibiotrophic fungus *Fusarium graminearum*. GLVs may thus constitute a promising agronomic tool. However, not much is known about the underlying priming mechanisms. Building further on these findings, we attempt to unravel the mechanism of priming by Z-3-HAC. Here, we used an untargeted metabolomics approach, to identify changes in the metabolome of wheat after exposure to Z-3-HAC and a subsequent infection with *F. graminearum*. Our analysis revealed a large upregulation of the production of glycosylated compounds upon Z-3-HAC treatment and a downregulation of the production of the benzoxazinoid DIMBOA. In addition to an untargeted metabolomics, we were interested in the response of a selection of several plant defense hormones and metabolites of the glutamate biosynthesis. We observed a large induction of salicylic acid biosynthesis, whereas other plant hormones were not affected by Z-3-HAC. Concurrently, we found a large decrease in components involved in glutamate metabolism.
Together, these findings illustrate that exposure to Z-3-HAC has both a large effect on primary N metabolism and induces glycosylation of metabolites, which may contribute to the increased defense.

Keywords: Volatile, priming, innovative crop protection, Fusarium, metabolomics, wheat

Session 5
Oral 40

Discovering coherency of plant reflectance, gene expressions and enzyme activities during different barley – powdery mildew interactions

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The Mildew locus o (*mlo*) gene based resistance of barley is successful against infestation by *Blumeria graminis* f.sp. *hordei* (*Bgh*), the causal agent of barley powdery mildew, by fast cell wall appositions (papilla). Modified epidermal cells remain intact. Contrary, barley *Mla* gene based resistance leads to a single cell or tissue hypersensitive reaction. These resistance responses are based on a cascade of gen-protein signaling.
The objectives of this study are to characterize resistance reactions against powdery mildew using a hyperspectral-imaging microscope and to uncover relevant wavebands for the transcriptional pathway.
Experiments were conducted with *H. vulgare* near isogenic lines cv. Ingrid wild type (WT), a *mlo* gene based resistant genotype and cv. Pallas *Mla* gene based resistant genotype. Hyperspectral reflectance was measured every 3 hours until 48 hours after inoculation (hai) with *Bgh* and daily measurements until 5 days after inoculation to assess early interaction sites. Parallel gene expression and enzyme activity profiling were used to determine barley pathogenesis and resistance stages.
Differences in the relevance among single wavebands for hyperspectral characterization of barley – powdery mildew interaction types were assessed using the Relief algorithm.
Significant changes of relevance occur at specific key moments of barley – powdery mildew interaction 12, 24, 48 and 72 hai. Further data driven analysis indicated simultaneous processes between changes in hyperspectral reflection and observed gene expressions and enzyme activity over time, respectively.
These results enable an exhaustive interpretation of plant hyperspectral reflectance in compatible and incompatible plant-pathogen systems for non-invasive plant phenotyping.

Keywords: Crop resistance, phenotyping, genotyping, hyperspectral imaging, *Blumeria graminis*

Session 5
Oral 41

Control of Immunity during legume Rhizobium symbiosis

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Rhizobium and Legumes establish a symbiotic interactions resulting in the formation of root nodules in which rhizobia fix nitrogen. It is generally presumed that rhizobia/legumes symbiosis evolved from pathogenic interactions. However, even when rhizobial populations reach very high densities in the nodules, they do not elicit visible defenses reactions. With the aim to better understand the molecular mechanism(s) that prevent(s) the development of defenses during rhizobia/legumes interactions, we studied the *symCRK* mutant of *Medicago truncatula* which develops defense reactions in nodules. Proteomics analysis was performed on fractionated nodules and the proteomes of both partners was characterized. The results suggest a potential involvement of ethylene in the defenses developed by this mutant. In agreement with a potential role for ethylene in the *symCRK* phenotype: i) ACC oxidase activity, which is responsible for ethylene synthesis, is higher in the *symCRK* nodules than in the WT ones ii) ethylene treatments on mature nodules mimic the *symCRK* mutation and iii) the inhibition of ethylene synthesis reduces the intensity of defenses developed in the mutant nodules. Together our results suggest that the ethylene signaling pathway has to be inhibited in mature nodules in order to prevent the development of defense reactions and that SymCRK plays a role in this inhibition. We are now further testing this hypothesis or the action of other defense hormones in control of the symbiotic immunity.

Key words: Symbiosis, Rhizobium, legume plants, immunity, ethylene.

Session 5
Oral 42

Characterization of Individual Receptor-Like Kinase Domains in IOS1 from Arabidopsis

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Oomycetes are devastating filamentous pathogens that impact ecosystems and agriculture. Our research aims at characterizing the molecular mechanisms that govern the establishment of disease in host plants. To sense the environment, plant cells possess more than 200 plasma membrane-spanning receptors, which are composed of extracellular leucine-rich repeats (LRRs) and an intracellular kinase domain. We previously identified the Arabidopsis receptor-like kinase "Impaired Oomycete Susceptibility 1" (IOS1), which downregulates abscisic acid (ABA) signaling and contributes to the infection success of filamentous, biotrophic pathogens such as oomycetes and the powdery mildew fungus (Hok *et al.*, 2011; Hok et al., 2014). The extracellular region of IOS1 is composed of LRRs and a domain, which shares similarities with malectin from animals. Animal malectins bind carbohydrates and participate in monitoring the glycosylation state of proteins during their transit in the endoplasmic reticulum (ER). We observed retention of IOS1 in the ER, which appears to be mediated through the malectin-like (ML) domain. Yeast two-hybrid screens with the extracellular IOS1 domain identified several proteins, among which some localize to the ER. Results from the screen and subsequent studies will be presented and discussed.

Hok et al. (2014). Plant Physiol. 166, 1506-1518.
Hok et al. (2011). Plant Cell Environ. 34, 1944-1957.

*L. Gioradano is supported by the Labex “Signalife” [#ANR-11-LABX-0028-01] and the INRA SPE department.

Key words: Arabidopsis, oomycete, receptor, malectin, endoplasmic reticulum, abscisic acid

Session 5
Oral 43

A RPP8-like R gene controls *Potato virus Y*-induced veinal necrosis in tobacco

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Potato virus Y is one of the most damaging virus on tobacco worldwide. Aggressive necrotic strains can lead to considerable yield losses. The *va* locus in tobacco conferring resistance to PVY was characterized [1]. However, PVY isolates overcoming *this* resistance and inducing necrotic symptoms were observed in several countries. In this context, it is important to find new *va*-independent resistance mechanisms.
Therefore, phenotyping tests were conducted on 163 varieties of the ITB collection. Resistance to PVY was estimated by symptom survey, and the screening was performed with different PVY necrotic strains, including one able to overcome *va*-resistance.
Ten cultivars that do not carry the *va* locus failed to show the typical vein necrosis infection phenotype. Positive ELISA tests showed a viral accumulation in these plants, despite the absence of necrotic symptoms. A different mechanism, involving tolerance to the necrosis induced by PVY is therefore involved.
Fine mapping on a F2 segregating population showed that this tolerance trait is inherited as a single recessive gene and allelism tests showed that eight out of ten varieties carry the same gene. Anchoring the linkage map to the tobacco genome physical map allowed the identification of a candidate gene (*RPP8*-like R gene) with a SNP polymorphism in those 8 cultivars. Functional assays using TILLING mutants in the *RPP8* gene confirmed the role of this candidate in tolerance to necrosis induced by PVY. The vein necrosis symptoms may be due to a *RPP8*-mediated Hypersensitive Response (HR) induced by PVY, although inefficient to impede viral propagation. Such systemic HR would not occur when *RPP8* is mutated in the tolerant varieties. Experiments are in progress to confirm this hypothesis.
This gene could be used in future tobacco breeding programs to minimize the impact of *va* resistance-breaking strains and to limit crop losses.

Keywords: potyvirus, tolerance, tobacco, vein-necrosis, R-gene

[1] E. Julio, J. Cotucheau, C. Decorps, R. Volpatti, C. Sentenac, T. Candresse & F. Dorlhac de Borne. (2014). A Eukaryotic Translation Initiation Factor 4E (eIF4E) is responsible for the "va" Tobacco recessive resistance to potyviruses. Plant Mol Biol Rep. DOI 10.1007/s11105-014-0775-4.

Session 5
Oral 44

Diversity in wheat Composite Cross Populations provides protection from the new yellow rust (*Puccinia striiformis*) races

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Composite Cross Populations (CCPs) display intra-specific diversity and allow for evolutionary breeding. Their genetic diversity allows them to adapt to the environmental conditions in which they are grown. An important question is whether CCPs can also deal with evolving pathogens such as the new yellow rust (*Puccinia striiformis*) races that have dominated most European countries after 2011. Three winter wheat CCPs, composed of 9, 12, or 20 parental varieties have been grown at the University of Kassel in both a conventional and organic system, without conscious selection since 2005 (F_5). For comparison, several current commercial varieties were grown every year in both systems. Disease pressure was still low in 2012 (mean AUDPC = 116) when the new races were not yet evident locally. Yellow rust dominated in 2014, 2015, and 2016 (mean AUDPC = 345, 438, 232, respectively). In these three years, mean AUDPCs in the CCPs were 332 and 334 in the reference varieties. AUDPCs varied by a mean of 54% among CCPs, while among reference varieties the variation was 130%. The modern commercial varieties, in comparison, were either very resistant or considerably more susceptible than the CCPs, most likely due to major genes and their genetic uniformity. These results indicate that the CCPs show low to moderate susceptibility to the new yellow rust races and that disease incidence was more predictable than among the modern varieties. This is despite the fact that many of the parental varieties that were grown in small plots in 2015, tended to be highly susceptible, having been bred between the 1930s and 2000, before these new races were present. The results illustrate the buffering capacity and potential of the genetically diverse CCPs to adapt to the changing biotic pressure of the yellow rust pathogen.

Keywords: Composite Cross Populations (CCPs), Evolutionary Breeding, Yellow rust, adaptation

Session 5
Oral 45

Developmental processes altered during gall formation by root- knot nematodes

Rocío Olmo[1*], Javier Cabrera[1*], Fernando E. Díaz-Manzano[1], Marta Barcala [1], Maria Fe Andrés[2], Ana Cláudia Silva[1], Isabel Martínez[1], Virginia Ruíz[1], Carmen Fenoll[1] and Carolina Escobar[1]

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Plant endoparasitic sedentary nematodes cause severe losses in different crops. Among the most harmful are the root-knot nematodes (RKNs; *Meloidogyne* spp.). RKNs enter by the root elongation zone and migrate intercellularly. They stablish within the vascular cylinder and induce specialized feeding cells, giant cells (GCs) from vascular cell precursors, still not well known, after repeated mitosis with partial cytokinesis. The cells around the GCs proliferate and the cortex cells hypertrophy, developing a gall, a newly formed pseudo-organ (Escobar et al., 2015).

We obtained the transcriptomes of early-developing GCs (at 3 days post inoculation) and galls induced by *M. javanica* in Arabidopsis using laser capture microdissection (Barcala et al., 2010). Interestingly, GCs and galls transcriptomes shared similarities to those from the quiescent centre and lateral root initial cells (Cabrera et al., 2014). Hence, we checked marker and mutant lines of key genes for both cell types, among others, lateral organ boundaries-domain transcription factors, *LBDs*, crucial during lateral root development, or *HSFB4 (SCHIZORRIZA)*, a ground tissue and quiescent centre marker.

Our results confirm that RKNs partially 'hijack' plant transduction pathways leading to the formation of lateral roots with common transducers as *LBD16 or mRNA390*, expressed in the proliferative cells within the galls and in the GCs (Cabrera et al., 2014; 2016), similarly to *HSFB4*, and to other key markers for root tip meristem. Moreover, the massive gene repression observed in the GCs, is partially regulated through miRNAs as miR172, crucial during flowering. This brings together apparently unrelated and distant processes such as flowering in the aerial parts, lateral root formation, or the root meristem maintenance in roots and morphogenetic responses to pathogens in roots. All these suggests that common regulatory molecular partners at the cellular level might control different organogenetic processes triggered either by internal developmental cues or by biotic interactions.

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Key words: Root knot nematodes, lateral root, giant cells, galls, root meristem

Session 6. EMERGING TOOLS FOR THE MANAGEMENT OF PLANT DISEASES: AGROECOLOGY AND DISEASE MANAGEMENT – part I

Keynote lecture 9

European Platform of research and development of tools for biocontrol of phytopathogens: SMARTBIOCONTROL

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Within the Interreg FWVL PHYTOBIO project (2010-2014) and more recently the new project portfolio SMARTBIOCONTROL (2016-2020), 26 partners from university laboratories, research centers or agricultural technical institutes and company decided to gather their skills to discover new biomolecules (mainly lipopeptides) retaining high potential to control efficiently, and in a sustainable manner, a large panel of crop diseases. In the PHYTOBIO project, a dozen of amphiphilic metabolites produced by bacteria, mainly belonging to the *Bacillus genus,* displayed promising activities as biocontrol agents, both in greenhouse and field conditions.

The new project portfolio SMARTBIOCONTROL is composed of 4 main scientific projects working on different fields of biocontrol in the cross-border region of France-Flanders-Wallonia:

- the **BioScreen** project aims at discovering new multifunctional molecules of biological origins able to either directly inhibit microbial pathogens and/or induce systemic resistance in the crops of interest.

- the **BioProd** project will optimize the production and purification of these molecules at pilot scale. In addition, the biodegradability and the toxicity of the new molecules will be monitored and many formulations will be assayed in order to get these molecules commercialized and delivered to farmers in the most stable, active and easy-to-handle forms.

- the **BioProtect** project will work to improve the use of biological crop protection products to combat phytopathogens in a sustainable way and to reduce the amount of chemical in crop protection. The project will also focus on the optimization of the efficacy of biological crop protection products in (field) trials.

- the **BioSens** project will develop a new generation of biochips allowing to track these biocontrol agents (microorganisms and/or biocidal molecules) to evaluate their persistence after application in the field.

In this keynote lecture, we will present i) the main results of the Phytobio project related to the screening of the bioactive molecules, their mode of action, their production process and their efficiency in greenhouses and fields trials and ii) the concept and strategy of the SMARTBIOCONTROL portfolio.

Keywords: SMARTBIOCONTROL, BIOSCREEN, BIOPROD, BIOPROTECT, BIOSENS, INTERREG V FWVL

Session 6
Oral 46

Development of a new biological control product for powdery mildew control in cereals

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Objectives

The objective of our research as part of the EU project BIOCOMES is to select new fungal antagonists for control of *Blumeria graminis* f.sp. *tritici* in wheat.

Methods used

More than 1200 fungal isolates were obtained from powdery mildew pustules on leaves collected in The Netherlands, Germany and Sweden. Potential candidate antagonists were selected by high-throughput screening methods followed by efficacy tests under controlled and field conditions.

Results

The pre-screening criteria were fulfilled by 748 isolates. These criteria were: no growth at 36°C (safety for humans), germination and growth at 5°C (cold tolerance), germination and growth at -7 MPa (drought tolerance) and growth after exposure to UVb (UVb tolerance). In a next step 27 isolates were excluded, because they belonged to species considered as not safe for humans, animals or plants. 185 representative isolates with high spore production were selected and their efficacy to reduce powdery mildew was assessed in bioassays on wheat seedlings (cv. Julius) conducted at 15°C and high humidity. Numbers of produced *B. graminis* conidia and the leaf coverages with powdery mildew pustules were quantified. The 10 isolates with highest efficacy and most consistent results were then tested on potted spring wheat plants (cv. Calixo) in the open field followed by an efficacy trial in a spring wheat crop (cv. Heron). The best isolates significantly reduced the leaf coverage with powdery mildew and the increase of the powdery mildew epidemic in time.

Conclusions

New antagonistic isolates were selected for the development of economically feasible biological control agents. Next research steps will focus on mass production and formulation, additional field testing and mode-of-action.

Acknowledgement

This project has received funding from the European Union's Seventh framework Programme for research, technological development and demonstration under grant agreement no 612713.

Session 6
Oral 47

Multitrophic control of Fusarium Head Blight: new acquisition

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Fusarium Head Blight (FHB) is one of the most important diseases of wheat, causing losses in yield and quality, this last due to mycotoxins production by the main causal agent *Fusarium graminearum* (FG). The early detection and control of trichothecene-producing *Fusarium* spp. is crucial to prevent toxins entering in the food chain. Biocontrol of FHB is a valid strategy and entails the treatment of crop residues and/or of spikes during anthesis with antagonists. The aim is to prevent the pathogen infecting plants and competition seems to be one of the main mechanisms of action.

Two beneficial fungi, *T. gamsii* 6085 (TG) - able to control *F. graminearum* growth and mycotoxin accumulation, and to reduce FHB in field - and *Fusarium oxysporum* 7121 (FO) – a good saprotrophic competitor for crop residues – have been used in a multitrophic approach in order to control *F. graminearum* growth (evaluated by Real-time PCR) and to reduce trichothecenes production (measured by HPLC) on natural substrates. In addition, Niche Overlapping among the two antagonists and the pathogen was further investigated by using the Biolog microarray system.

The two beneficial fungi are able, to a different extent, to reduce pathogen growth and mycotoxins accumulation in natural substrates. No antagonistic effects has been registered between the two antagonists when used together, leaving open the possibility to use them in a multitrophic approach that will increase the probability of success of the disease control. Biolog results show that FG and FO occupy the same niche and compete for nutrients. FG dominates on TG suggesting that nutrient competition is not the main mechanism of action for TG.

Finally, thanks to the availability of the genomes of both isolates, and the genome-editing (CRISPR-Cas) protocols, we are actually collecting new information and attempting to improve the efficacy of the two antagonists towards FHB.

Keywords: Fusarium Head Blight, *Fusarium graminearum*, *Trichoderma gamsii*, *Fusarium oxysporum*, mycotoxins, multitropic approach, genome editing

Session 6
Oral 48

Cell wall glycomolecules and border cells: sentinels for root defense against soil-borne pathogens.

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Most plant species release thousands of living root border cells in the rhizosphere (Hawes et al., 2000). Due to their very special position at the interface between roots and soil, border cells are key elements involved in root protection against soil-borne pathogens.
In pea (*Pisum sativum*), root border cells are specialized in the production of anti-microbial compounds including pisatin, arabino-galactan proteins (AGP) and extracellular DNA (Cannesan et al., 2011; 2012; Driouich et al., 2013). Root border cells and their associated mucilage were shown to be essential in shaping soil-borne microbial communities surrounding the root tip (Nguema Ona et al., 2013). We have assessed the impact of AGP produced by pea root cap and border cells on the development cycle of *Aphanomyces euteiches*, a pathogen causing root rot disease. *In vitro* assays have revealed that AGP attract the zoospores, induce their encystment and prevent their subsequent germination. These data provide evidence for a role of AGP in root-microbe interaction (Cannesan *et al*., 2012). We have also shown that root border cells from different plant species secrete extensin, another hydroxyprolin-enriched glycoprotein belonging to the same family as AGP (Plancot et al., 2013; Koroney et al., 2016). In addition, extensin secretion seems to be enhanced in response to elicitors (Plancot et al., 2013, Castilleux et al, unpublished).
We proposed a model termed "Root Extracellular Trap" or RET in which we postulated that root mucilage together with root border cells could function in root defense in a way similar to that of neutrophil extracellular traps (NET) in mammalian cells (Driouich *et al*, 2013). Cell wall glycomolecules including callose, AGP and extensin appear to play a critical role in root immunity and plant health. In this communication, we will present and discuss the functional organization of root border cells and their secretions in plant defense.

Acknowledgments : This work was supported by Le Grand Réseau de Recherche VASI « Végétal-Agronomie-Sols et Innovations ».

Keywords: arabinogalactan-proteins, cell wall, extensin, oomycetes, root border cells

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Session 6
Oral 49

Resistance induced by ulvans against plant pathogenic fungi

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Ulvans are water-soluble sulfated heteropolysaccharides extracted from the cell walls of green marine macroalgae *Ulva* spp. and are able to induce systemic resistance in plants. The aim of this report is to summarize the main results of our research group, from discovery to attempts in elucidating the mechanisms of action. A bioprospecting study carried out between 2002 and 2004 identified *Ulva fasciata* Delile as a potential source of active compounds for controlling plant diseases. In greenhouse, spray of *Ulva* extracts reduced (about 80%) the number of powdery mildew colonies on pre-treated leaves. When applied under field conditions, sprays of the algal extracts were also able to control anthracnose by 50% in a highly susceptible bean cultivar. Follow-up studies revealed that ulvans were the main elicitor molecules in the extracts of *Ulva*. In greenhouse, ulvans demonstrated to protect several crop plants such as beans, apple, grapevine and cereals (e.g. wheat and barley) against a broad range of foliar phytopathogens, including biotrophic, hemibiotrophic and necrotrophic fungi. Pretreatment of barley leaves with ulvans significantly reduced the severity of *Blumeria graminis* by 80%, and in cereals, priming effect was detected in assays with suspension-cultured cells. Resistance induced by ulvans reduced the bean anthracnose severity by 50%, lasting at least up to nine days after treatment. Protection levels of 65% were recorded in ulvans-treated apple plants against the Glomerella leaf spot, but the effect was weaker in not-directly treated distal parts. Jasmonic acid pathway as well the enzyme NADPH oxidase were triggered during ulvan-induced resistance in *Arabidopsis thaliana*. These findings may open new horizons for using the algal polysaccharides as resistance inducers.

Keywords: seaweeds, anthracnose, powdery mildew, ulvans, induced resistance.

Session 6
Oral 50

A Combined use of Biostimulants and Resistance Inducers to improve wheat protection

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In the context of the Ecophyto French National Action Plan, France is committed to reduce gradually the use of pesticides on agricultural crops while maintaining a high production rate and good food quality. As an alternative to pesticides, known to act directly on pathogens, new elicitors, or Resistance Inducers (RIs), are being developed to protect plants *via* the stimulation of plant defenses. Generally the efficacy of RIs to protect plants is now well established in controlled and greenhouse conditions, but may substantially decrease when applied to crops in field conditions. We aim here at combining RIs with biostimulants (BSs), in order to increase their protective efficacy by optimizing the physiology of treated plants. Despite their growing interest in agriculture, the modes of action of BSs on plants have been poorly characterized so far. In the IRIS+ project, we focus on one major crop of great economic interest: bread wheat (*Triticum aestivum* L.) to (i) evaluate the impact of three new molecules considered as BSs on plant growth and physiology and (ii) screen a set of five new molecules considered as potentials RIs against two foliar cryptogamic diseases: powdery mildew (PM, caused by *Blumeria graminis* f sp. *tritici*) and *Septoria tritici* blotch (STB, caused by *Zymospetoria tritici*), (iii) select at least one efficient BS/RI combination in order to improve the level of wheat protection against the two diseases, compared to a treatment with a RI alone. Among the five RIs tested to protect wheat against PM and STB, SDN2, SDN3 and SDN5 were efficient against PM, with protective effects of 20-30%, 20-40% and 65% respectively. Moreover, SDN3 and SDN5 were also efficient against STB, they decreased necrotic leaf area by 50% and almost 80% respectively, and also reduced sporulation by 30-70% and 40% respectively. The three BS have been tested in different conditions (methods of applications, substrate, nutrition) for their effect on plant growth parameters and leaf pigment content, but no global positive effect could be detected. The direct activity of RIs and BSs on the two fungus strains have also been tested *in vitro*: SDN2 weakly inhibited *B. graminis* spores germination, SDN3 totally inhibited *Z. tritici* mycelium growth and SDN5 totally inhibited *B. graminis* spore germination and strongly delayed *Z. tritici* mycelium growth. We still have to determine whether the protective effects of RIs obtained *in planta* are due to the stimulation of wheat defenses, direct activity on *B. graminis* and *Z. tritici*, or a combination of both modes of action. Concerning BSs, we have to optimize a method of application relevant to an evaluation of physiological and growth parameters before considering a combined use of RIs and BSs.

Keywords: Bread wheat, Powdery mildew, Septoria tritici blotch, Resistance inducer, Biostimulant

Session 6
Oral 51

Burkholderia genome mining for NRPS reveals a great potential for lipopeptide production with biocontrol applications

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Bacteria belonging to the genus *Burkholderia* live in various ecological niches and present a significant role in the environments through the excretion of a wide variety of modular secondary metabolites including non ribosomal peptides (NRPs) and polyketides (PKs). These secondary metabolites represent an important source of natural products that can be used to promote plant growth or replace chemical pesticides as they have antifungal activities together with biosurfactant properties.

A genome mining was performed on 48 fully sequenced genomes of *Burkholderia* species publicly available. This *in silico* screening for new secondary metabolites, using specific bioinformatics tools, revealed a total of 161 clusters containing nonribosomal peptide synthetases (NRPSs) and predicted the synthesis of at least 11 novel products. Most of them are siderophores or lipopeptides, two classes of compounds with potential application in biocontrol. A dozen of synthetases harbouring signatures for cyclic lipopeptide production (presence of Cstarter, and dualC/E domains for epimerisation of corresponding monomers) were predicted from genome sequences of the single strain, displaying the ability to decrease surface tension to 27 mN m^{-1}. Consequently, this strain is considered as a very good candidate for biocontrol agent development. Other lipopeptides like burkhomycin and burkholdin, attractive for its antifungal activity, were shown to be produced by various strains.

Keywords: *Burkholderia*, Biocontrol, lipopeptides, NRPS, genome mining

Session 6. EMERGING TOOLS FOR THE MANAGEMENT OF PLANT DISEASES: AGROECOLOGY AND DISEASE MANAGEMENT – part II

Keynote lecture 10

RNA-based control of fungal diseases: application and mechanisms

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RNA interference has emerged as a powerful genetic tool for scientific research. The demonstration that agricultural pests, such as insects and nematodes, are killed by exogenously supplied RNA targeting their essential genes has raised the possibility that plant diseases can be controlled by lethal RNA signals. We have shown that transgenic expressing[1] (Host-Induced Gene Silencing) or spraying[2] (Spray-Induced Gene Silencing) a 791 nt long double-stranded (ds)RNA (termed *CYP3*-dsRNA) that targets the three essential fungal ergosterol biosynthesis genes (*CYP51A*, *CYP51B*, *CYP51C*), efficiently inhibited the necrotrophic fungus *Fusarium graminearum on barley*. Strong inhibition of fungal growth required an operational fungal RNA interference mechanism as demonstrated by the fact that a Fusarium DICER-LIKE-1 mutant was insensitive to *CYP3*-dsRNA. Further analysis showed that *CYP3*-dsRNA was systemically translocated in the plant via the phloem and efficiently taken up by the fungus in distal, non-sprayed leaf areas. Consistent with this finding, it was recently shown that spray applications of RNA to plants, including Arabidopsis, tomato and tobacco, is rather effective in controlling plant diseases caused by microbial pathogens.[3] Moreover, optimization of RNA delivery by certain nanostructure-based formulations has been shown.[4]
I will discuss recent developments in the use of RNA as a new chemical for the control of plant diseases.

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Keywords: Fusarium, small RNA, DICER, HIGS, SIGS

Session 6
Oral 52

Towards agroecological management of rice blast disease in Madagascar Highlands

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Rice is the staple crop and food in Madagascar. Since the 1980s, a joint breeding program conducted by CIRAD and Fofifa, has led to the creation of new varieties adapted to the high altitude and at the same time to rainfed cropping environment (Raboin et al., 2014). But the first released varieties rapidly became susceptible to rice blast, a fungal disease caused by *Magnaporthe oryzae,* and had to be abandoned. In the 2000 and 2010s, new varieties tolerant to blast were proposed to farmers. Upland rice cropping system has become an important complement to irrigated rice, and a way to improve self-sufficiency in rice and food security. Rice blast, a fungal disease caused by *Magnaporthe oryzae,* is a major constraint for rice particularly in upland cropping conditions. In developing countries like in Madagascar, no chemical solution can be considered, so there is a crucial importance to find out agroecological solutions to limit the risk of epidemics. The interactions between upland rice and blast epidemics have then been studied in a multidisciplinary team in the highlands of Madagascar for more than 10 years. Conservation agriculture cropping systems were studied (Sester et al., 2014, Dusserre et al., in press) and showed an interesting impact on rice susceptibility to blast. Complementary field experiments showed that rice susceptibility was affected by soil origin and crop density and that infested crop residues appeared as sources of primary inoculum (Raveloson et al., 2013). Experiments on cultivar mixtures showed promising results to control blast epidemics (Raboin et al., 2012). These data were measured in field experiments and integrated together in a simulation model at the landscape scale (Sester et al., 2016).

Key words: blast, Rice, Agronomic Management, Food security

Session 6
Oral 53 : canceled and converted into Poster 168

Mosaics of plant disease resistance genes are a more versatile means of achieving disease control than pyramids in most agricultural landscapes

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The breakdown of plant virus resistance genes is a major issue in agriculture. We investigated whether a set of resistance genes would last longer when stacked into a single plant cultivar (pyramiding) or when deployed individually in regional mosaics (mosaic strategy).

We modeled the genetic and epidemiological processes shaping the demo-genetic dynamics of viruses under a multi-locus gene-for-gene system, from the plant to landscape scales. The landscape consisted of many fields, was subject to seasonality, and of a reservoir hosting viruses year-round.

Strategy performance depended principally on the fitness costs of adaptive mutations, epidemic intensity before resistance deployment and landscape connectivity. Mosaics were at least as good as pyramiding strategies in most production situations tested. Pyramiding strategies performed better only with slowly changing virus reservoir dynamics. Mosaics are more versatile than pyramiding strategies, and we found that deploying a mosaic of three to five resistance genes generally provided effective disease control, unless the epidemics were driven mostly by within-field infections.

We considered the epidemiological and evolutionary mechanisms underlying the greater versatility of mosaics in our case study, with a view to providing breeders and growers with guidance as to the most appropriate deployment strategy.

Keywords: Durable disease resistance, Pyramids of plant resistance, Mosaic of plant resistance, Landscape epidemiology, Regional deployment strategy

Session 6
Oral 54

Risk assessment and management of Banana streak viruses in Guadeloupe

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Several species of Banana streak virus (BSV) occur in banana. They cause a wide range of symptoms on leaves, pseudostem and fruits, although the impact of infections on yield and fruit quality has never been properly assessed. BSVs are naturally transmitted by mealybugs. However, spontaneous infections occur in interspecific varieties such as plantains, following stress-induced activation of infectious endogenous BSV sequences (eBSVs) integrated in B (*Musa balbisiana*) genomes.

The kinetics of activation of infectious eBSVs was monitored in Guadeloupe in an experimental plot, using a random block design. It showed that infectious eBSVs display differential activation potentials in plantain varieties French Clair and Pelipita, pointing to a role of plant genetic background in the activation process. It also showed that the multiplication mode of planting material influences activation levels monitored under field conditions and that infection had no significant impact on plant growth and fruit production of both varieties.
A wide range prevalence study of BSVs undertaken throughout Guadeloupe‘s plantations, Creole gardens, abandoned fields and wild areas among varieites representative of the main dessert banana and plantain types grown in Guadeloupe showed that overall BSV prevalence were low in dessert banana and cooking banana. Compared with a similar survey carried out in 2006, prevalence was very similar for dessert banana but significantly lower for plantains, which carry eBSVs that interfere with molecular diagnostic and cause frequent false positives. It is likely that the recent optimization of BSV molecular diagnostic increased the accuracy of detection.

Overall, these results suggest that BSVs have a low prevalence and unmeasurable impact on dessert banana and plantain in Guadeloupe, owing to low vector-borne transmission and low activation of infectious eBSVs. These results also lead to recommendation regarding the management of BSVs through safe multiplication modes of plantain planting material.

Keywords: Banana streak viruses; endogenous viral elements; activation; prevalence; risk assessment

Session 6
Oral 55

INATREQ™ ACTIVE – a new fungicide for control of Septoria tritici blotch (*Zymoseptoria tritici*)

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Septoria tritici blotch (STB) (*Zymoseptoria tritici*) is a serious and very common pathogen in wheat crops. Inoculum is present in all intensive wheat producing countries and the level of severity depends on humidity and rain events during the growing season. Between 1 and 4 fungicide treatments are commonly targeting control of STB per season in Western Europe depending on the actual risk. In recent years the control of STB is challenged following resistance development to both strobilurins and triazoles. More recently isolates with reduced sensitivity to SDHI's in UK and Ireland have also been detected. Of additional concern in the coming years is the possibility that some molecules that are widely used today will be under regulatory pressure and may exit the market.

Due to the high incidence of fungicide resistance in *Z. tritici*, it is seen as very positive, that a new fungicide – Inatreq™ Active (fenpicoxamid) with a unique biochemical mode of action at a new target site in this segment has been discovered by Dow AgroSciences and should be available to cereal growers in 2019/2020. The new active is derived from a natural compound produced by fermentation of an Actinomycete (*Streptomyces spp.*) which then undergoes a minor alteration to stabilize the product. Inatreq shows no cross-resistance to existing cereal fungicides, including triazoles, strobilurins and SDHIs. However, as the active in Inatreq is a target site inhibitor, the product should only be used in combination with other actives to minimize the risk of resistance development.

Inatreq has been tested in early development trials in Denmark and France. These trials have consistently confirmed excellent control of STB and yield benefits under both preventive and curative conditions when applied as a T1 or T2 spray. Inatreq's strong residual effect and curative activity on STB have been shown to offer flexibility in dose and application timing. In Denmark, strategies with Inatreq have given significantly better control of STB as well as significantly better yields compared to all existing strategies.

Keywords: disease management, resistance management, wheat**,** Septoria tritici blotch, Inatreq

Session 6
Oral 56

Management of hairy root disease (crazy roots) in tomato

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The hairy root disease, often termed 'crazy roots', has in recent years become a serious problem in greenhouse hydroponic cultures of tomato, eggplant and cucumber in many European countries. The typical symptoms include excessive development of roots, which leads to more pronounced vegetative growth of the plants and significant losses of marketable yield. The project consortium working on the crazy roots topic aims to find integrated management solutions to tackle this problem. An overview of their results so far will be presented.

In a first step, isolates of rhizogenic *Agrobacterium* were collected from different growers, establishing a collection of over 100 isolates. The isolates were subsequently genotypically and phenotypically characterized, which indicated the highly complex nature of this pathogen. Subsequently, the effect of various cultural methods was tested, such as the use of disinfectants and choice of substrate. In addition it was observed that the persistent nature of the *Agrobacterium* strains is in many cases due to their ability to form biofilms in the irrigation system, adding another layer of complexity to their control. Finally, a screening was also performed to identify possible biological control organisms against rhizogenic *Agrobacterium.* The knowledge developed within the consortium has been applied into practice at different growers with promising results.

Keywords: rhizogenic *Agrobacterium*, crazy roots, tomato

SESSION 1. FROM PLANT-MICROBE INTERACTIONS TO INTERACTIONS WITHIN PHYTOBIOMES

Session 1
Poster 1

BestPass: boosting plant-endophyte stability, compatibility and performance across scales

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Faced with multiple challenges, global agriculture must adopt more dynamic, efficient and sustainable production methods to increase food and fodder production to feed a growing population with fewer resources (FAO).
The BestPass project is a Marie Curie "Early training network" with 15 PhD students hosted by 12 beneficiariesa plus 7 partner organisationsb. The 15 PhD projects concern endophytes in plants. Model plants include tomato, Salicornia and grasses (*Festuca* and *Lolium*) and both bacterial and fungal endophytes including both newly discovered and model organisms. The projects range from fundamental studies of the biology of endophyte-host interactions to understanding the factors underlying the development of stable microbial products that can boost plant performance.
a Beneficiaries: ABiTEP, ACIB, Austrian Institute of Technology, Aarhus Univ, BOKU, CSIC, DLF, IGZ, INOQ, NCU, TU Graz, Univ Amsterdam as well as UCPH
b Partner Organisations: Universities: Cologne, Massey, Michigan State and Wageningen, as well as SMEs: Biotenzz, Biofungitek, Roombiotic
The project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 676480.

Session 1
Poster 2

Microbiome diversity in crop debris and potential interactions with the fungal pathogens *Zymoseptoria tritici* and *Leptosphaeria maculans*

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Despite the recognized contribution of crop debris as inoculum source of many plant pathogens, little is known about the structure and function of microbial entities associated to this plant habitat, and their interactions with residue-borne plant pathogens.
The objective of this work is to characterize bacterial and fungal assemblages associated to wheat and oilseed rape debris and to assess their relative impacts on the build-up of primary inoculum of *Zymoseptoria tritici* (septoria leaf blotch of wheat) and *Leptosphaeria maculans* (stem canker of oilseed rape) during the inter-cropping period.
This two-years study is based on a field design composed of three plots (wheat monoculture, oilseed rape-wheat rotations) established in the experimental domain of Grignon (Paris basin). Wheat and oilseed rape crop debris were sampled four times a year during the ascospore release period (from October to May). The structure of debris-associated microbial assemblages was assessed through a combination of classical microbiological approaches and MiSeq sequencing of two molecular markers (16S rRNA gene and ITS1 region of the fungal internal transcribed spacer).
Community profiling approach was performed on approximately 100 samples collected on the first year of the experiment. After suppressing rare OTUs (Operational Taxonomic Units), around 110 bacterial OTUs and 70 fungal OTUs were identified per sample. According to beta diversity analysis, the crop (wheat, oilseed rape) was the main, significant factor structuring debris-associated microbial assemblages. The sampling date was also shaping a microbial community composition.
These first results, which will be combined to those of the second year of sampling, reinforce our view on the community composition, and give an opportunity to identify microbial species potentially acting on primary inoculum of *Z. tritici* and *L. maculans.*

Keywords: *Zymoseptoria tritici*, *Leptosphaeria maculans*, microbial diversity, plant debris, primary inoculum, metabarcoding

Session 1
Poster 3

Screening of wheat endophytes as biological control agents against *Fusarium* head blight

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Fusarium head blight (FHB), caused by phytopathogenic fungi such as *Fusarium graminearum* and *Fusarium culmorum*, is one of the most important diseases on wheat crops worldwide. The aim of the present study was to find endophytes of wheat candidates as biological control agents (BCAs) against FHB, using two different *in vitro* tests. The common *in vitro* test by dual culture assays was used to conduct a large-scale screening of 86 strains, isolated from wheat plants, towards *Fusarium* spp. In addition, an *in vitro* screening test on detached wheat spikelets, easy to handle but closer to real life, has been developed on a subselection of 22 strains. Both *in vitro* tests identified 13 strains promising for the control of FHB, of which 10 belonged to three species not reported before for their antagonistic capacities against *Fusarium* spp. However, the efficacy of some strains turned out different between both *in vitro* tests, raising the importance of finding the most appropriate screening approach for the search of BCAs. This study pointed out the interest of the test on detached wheat spikelets that provided information about a potential pathogenicity, the growth capacity and efficacy of the endophyte strains on the targeted plant, at early screening step.

Keywords: *Fusarium*, biocontrol, endophytes, screening, real-time PCR

Session 1
Poster 4

Fungal endophyte diversity in chestnut galls and surrounding tissues in plots with different tree composition

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Fungal endophytes are potential bio-control agents for pest insects. Yet, their diversity and functions need further understanding. We explored fungal endophyte diversity in galls induced by an invasive insect, *Dryocosmus kuriphilus*, and in surrounding chestnut leaf tissues, sampled in mature forest plots where chestnuts where growing alone or mixed with pines, oaks or ashes. We hypothesized that endophytes communities in galls differs within plot composition and that galls tissues shelter a more diverse and rich endophyte community than leaf tissues.
We selected 28 chestnut plots consisting in 10 chestnut monocultures and 18 two-species mixtures. In each site, we sampled galls and leaf tissues (3 samples per tree and 3 trees per site of each type). We disinfected leaf and gall samples and extracted DNA. Fungal endophytes were characterized by Illumina sequencing of the Internal Transcribed Spacer 1 (ITS1) region.
We found a total of 1,378 different OTUs in our samples. The most common OTU corresponded to *Gnomoniopsis sp.* which may be associated to *D. kuriphilus* gall necrosis. Endophyte community richness and diversity on chestnut leaves and galls were independent of plot composition. Endophytes richness in gall tissues was reduced compared to surrounding leaf tissues.
These results suggest that plot composition do not impact endophyte communities from chestnut galls and that, interestingly, galls, commonly thought as a source of nutriment which can directly or indirectly attract fungal endophytes and act as a filter for fungal endophytes from chestnut leaves. A better understanding of their functioning is important to improve biocontrol agents for galling insects.

Keywords: endophytes, forest diversity, plant-fungus-insect interactions, chestnut

SESSION 2. SPACE-TIME AND MULTI-SCALES APPROACHES: DIAGNOSTIC, EPIDEMIOLOGY AND ECOLOGY IN THE FIELD

Session 2
Poster 5

AvrLm7 and *AvrLm3* frequency evolution in French *Leptosphaeria maculans* populations: a 20 year's survey

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Phoma stem canker, caused by the fungus *Leptosphaeria maculans*, is mainly managed through the deployment of resistant varieties. Specific resistance genes (*Rlm*) are present in commercial varieties and their effectiveness is a function of the frequency of the corresponding avirulence allele in field populations of the pathogen. The durability of a given *Rlm* gene may vary, depending on the plant genetic background (Brun et al. 2009), the fitness cost linked to the loss of the avirulence gene (Huang et al. 2010) and agronomic practices (Daverdin et al. 2012). After the very rapid breakdown of *Rlm1* in the 90's in France, it was questioned whether all released *Rlm* genes could be overcame at the same speed.
Rlm7 was introduced in commercial hybrids in France when most of the isolates possessed the avirulent allele *AvrLm7* (Balesdent et al. 2006). The frequency of virulent isolates was monitored in *L. maculans* populations of at a national scale (8 to 20 sites per year) from 2000 to 2015.
While only one virulent isolate toward *Rlm7* ("*avrLm7"*) was found in 2000, their mean frequency reached 4% in 2010, 20% in 2013 and 41% in 2015. Regional variations were observed. For instance in 2013, *avrLm7* frequency varied from 0% (Britany) to 45% (Center region). Due to the negative interaction between AvrLm3 and AvrLm7 (Plissonneau et al. 2016), nearly all *avrLm7* isolates were avirulent toward *Rlm3*. Only 1.8% of the current French *L. maculans* population can infect both *Rlm3* and *Rlm7* varieties.
Compared to the *Rlm1* breakdown that happened in only 3 growing seasons in France, 10 years of intense use of *Rlm7* were needed to reach a mean frequency of virulent isolates of only 20% at the national level. This survey also confirms the negative interaction between *AvrLm3* and *AvrLm7*, which offers great perspectives for durable management of *Rlm* genes in oilseed rape.

Keywords: *Leptosphaeria maculans*, resistance breakdown, *Rlm7*, *Rlm3*, avirulence genes, *Brassica napus.*

Session 2
Poster 6

Quantitative analysis of distribution of *Fusarium graminearum* and *Microdochium* spp. in winter wheat

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Because similar symptoms of plant disease can be produced by *Fusarium* and *Microdochium* causal agents, the use of symptoms alone is an inadequate method for disease identification in fields. Extensive colonization of leaf tissue and heads of winter wheat was occurred in 2016 in South region of Russia. The necrotic lesions of flag leaf and pink colored sporulation of fungi on head *have been observed* in many fields.

The quantitative PCR was used for analysis of fungal DNA content in different tissue (head, grain, peduncle, flag leaf, and stem) of wheat. Deoxynivalenol (DON) amounts in plant tissues were analyzed by ELISA.

F. graminearum was presented in all parts of the wheat plants of the Zadoks stage 85 (the content of fungal DNA varied in limits 1.4-74.0×10^{-7} ng/ng of total DNA). The large amounts of *F. graminearum* DNA contents were found in heads.

A significant positive correlation between the amounts of fungal DNA and DON in plant tissue was found ($r = +0.96$, $p<0.001$). The stems and peduncle of all analyzed wheat cultivars had lower DON content in compare with the other plant tissues.

In the all analyzed plant tissues the DNA of both *M. nivale* and *M. majus* have been found. Totally, content of *M. majus* DNA was significantly higher (from 8.5×10^{-4} to 1.2×10^{-1} ng/ng of total DNA), than *M. nivale* (from 8.1×10^{-5} to 3.6×10^{-3} ng/ng of total DNA) in all parts of vegetative plants. The flag leaves contained the maximum of DNA of both *Microdochium* species. A significant positive correlation between the contents of DNA of *M. nivale* and *M. majus* was found ($r = +0.57$, $p<0.05$). The species diversity, resistance of wheat cultivars and steps to minimize fungal infection in field are discussed.

The investigation was supported by the Russian Science Foundation (No. 14-26-00067).

Keywords: fungi, *Fusarium*, *Microdochium*, winter wheat, organs, real-time PCR

Session 2
Poster 7

Modeling impacts of fertilization on epidemics caused by a hemibiotrophic fungal pathogen

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A modeling framework focused on life history of foliar plant pathogens is being developed to simulate within and between season interactions between crop growth and disease development. It is a resource-based model relying on a physiologically structured population approach. The simulated pathosystem is, in particular, able to respond to both plant architecture and fertilization. The implementation takes place at the two-complementary patch and canopy spatial levels. A patch is a small infectable unit of leaf tissue (about the size of a mature infection or "lesion"). Production of new patches over time is a function of the available resource produced by the other patches depending of parameters such as photosynthetical activity and disease intensity. The pathogen propagules can contaminate nearby healthy patches. The definition of patch proximity depends on the dispersal ability of the pathogen which is determined by parameters such as dispersal mode (splash or wind) and canopy density. This framework is used to study spatiotemporal changes of a pathosystem with a hemibiotrophic fungal pathogen, namely *Zymoseptoria tritici* which causes Septoria tritici blotch on wheat. This disease is characterized by a complex infection cycle consisting of a long symptomless incubation period after the infection, followed by chlorosis and necrosis symptoms before fungal sporulation. A sensitivity analysis over fertilization parameters (i.e. patch resource carrying capacity, patch green lifespan, and maximum patch creation rate) is being performed in order to quantify the effects of crop resource level on plant physiology and disease dynamics. Such a resource-based modeling framework using an ecological approach studying the structure, function and dynamics of a system to address epidemiological questions is currently missing in the phytopathological community. This kind of tools may be very valuable to design more resilient agroecosystems, predicting pathogen dynamics under different fertilization conditions.

Keywords: Septoria tritici blotch, fertilization, epidemiology, life history theory, resource-based model, structured population model

Session 2
Poster 8

Biomarkers for Ramularia leaf spot disease in barley

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Ramularia collo cygni (Rcc), a hemibiotroph and close relative of *Z. tritici* is a pathogen of barley in Europe and South America. It causes the disease, Ramularia leaf spot (RLS), and involves the secretion of a photoactive toxin, rubellin, in the plant apoplast leading to necrotic leaf lesions and eventually up to 35% yield losses. *R. collo-cygni,* a seed-born fungus grows as an endophyte in the early developmental stages, therefore no complete resistance is available in barley germplasm and fungicides are proven to be inefficient due to the symptomless infection. Molecular mechanisms underlying the disease establishment and the switch between endophytic and pathogenic status are currently unknown.

My PhD project focuses on the identification of key gene-protein families and their function in barley-*Ramularia collo-cygni* interplay using "omics" approaches. I am deploying the promising candidates for disease tolerance to develop biomarkers based on promoter/gene sequences. Alternatively, I am using rubellin toxin to design an antibody to develop a diagnostic approach to detect the fungus in the barley cultivars at different developmental stages.

Transcriptomics and proteomics analyses of two contrasting (tolerant vs susceptible) barley cultivars were performed to identify the active genes/proteins and pathways during different stages of the disease establishment. Preliminary results from the transcriptome analyses allowed us to pinpoint specific gene families, biological and molecular functions (receptors, transporters, transcription factors) are differentially regulated between cultivars, suggesting their major role in RLS disease in barley. Candidates identified and confirmed from omics data analyses will be used as potential candidates for genetic marker development in the plant breeding programs. We also found that different barley cultivars respond differently to rubellin treatment indicating the involvement of cultivar specific barley gene(s). Together all these results with will be presented at the conference.

Keywords: *Ramularia collo-cygni*, Ramularia leaf spot disease, rubellin, omics, biomarker.

Session 2
Poster 9

Molecular variabilty and genetic structure of IYMV in Burkina Faso

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Imperata yellow mottle virus (IYMV, Sobemovirus) was first described in 2008 in the south-western region of Burkina Faso (West Africa). The genetic diversity of IYMV was not documented up to day. In this study, the variability of CP of IYMV was evaluated through the molecular characterization of 39 isolates collected in the western part of Burkina Faso. Comparison of sequences of these new isolates and one IYMV sequence available in GenBank revealed that the average nucleotide diversity was low for a plant virus. The ratio of nonsynonymous over synonymous nucleotide substitutions per site was low, indicating a CP diversification under strong purifying selection. In spite of the low nucleotide diversity, phylogenetic analysis revealed segregation of IYMV isolates into six major clade. Study of spatial distributon and genetic structure revealed no correlation between genetic variation and geographical origin of IYMV isolates. This is the first study of the genetic diversity of IYMV.

Keywords: Imperata yellow mottle virus (IYMV)-Coat protein(CP)-genetic variability-Genetic differentiation

Session 2
Poster 10

Coexistence of *Leptosphaeria maculans* and *L. biglobosa* on oilseed rape crops

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Leptosphaeria maculans and *L. biglobosa* are associated with phoma stem canker of oilseed rape. In many countries, *L. maculans* is considered to be more damaging than *L. biglobosa*, which is generally associated with upper stem lesions. However, *L. biglobosa* is an important pathogen in Poland and in China the disease is associated only with *L. biglobosa*. This work studied the coexistence of *Leptosphaeria* spp. on winter oilseed rape crops over three to five cropping seasons in Germany, the UK and Poland. The relative contribution of the two *Leptosphaeria* spp. to phoma leaf spot and phoma stem canker severity was examined on cultivars differing in their resistance against *L. maculans*, including cultivars with the *Rlm7* resistance gene. There was extensive colonisation by *L. biglobosa* on cultivars with the *Rlm7* gene. Effective control of *L. maculans*, by using cultivars resistant against this pathogen, may increase the possibility of severe epidemics caused by *L. biglobosa* in the future. Combined data for the abundance of the two *Leptosphaeria* spp. in air-borne inoculum and their contribution to disease severity showed that *L. biglobosa* has an increasingly important role in development of disease epidemics.

Keywords: phoma stem canker, *Leptosphaeria maculans*, *Leptosphaeria biglobosa*, oilseed rape

Session 2
Poster 11

Death of the assumption that 'latent period' is fixed over the course of a plant disease epidemic

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The latent period is defined as the time between infection and the onset of sporulation due to that infection. It is a crucial life history trait, particularly for polycyclic diseases, because it determines how many complete infectious cycles can occur during an epidemic and its final intensity. Many studies have focused on the variation of latent period with pathogen or host genotype, or its plasticity in response to environmental factors. The focus on these aspects is unsurprising, as these factors form the apices of the epidemiological triangle (host, pathogen, and environment). Experiments in controlled conditions are generally used to assess pathogen pathogenicity (virulence and aggressiveness) and host susceptibility. Once estimated for one or several pairs of host-pathogen genotypes, the value of the parameter 'latent period' is implicitly considered to be fixed and is used in epidemiological models. Paradoxically, most epidemiological studies do not consider the latent period of a pathogen population to be intrinsically variable. My thesis here is that the latent period displays non-negligible variation over the course of a disease epidemic, and that this variability has multiple sources, some of which have antagonistic impacts. I develop arguments for four sources of variability challenging the implicit assumption that the latent period remains constant: daily fluctuations in leaf temperature, the nature of the inoculum, host stage or the age of host tissues, intra-population competition and selection for aggressiveness traits. I focus on experiences with *Septoria tritici* blotch (*Zymoseptoria tritici*), making use of empirical datasets collected during various research projects.

Keywords: generation time, latent period, plant disease epidemiology, polycyclic disease, wheat, *Zymoseptoria tritici*

Session 2
Poster 12

Characterization of German *Plasmodiophora brassicae* populations and possible strategies to suppress the clubroot disease in oilseed rape fields

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Clubroot caused by *Plasmodiophora brassicae*, is one of the most economically important diseases of oilseed rape (OSR) and other brassica vegetables worldwide. Disease outbreaks have caused serious problems in recent years in Germany. Therefore, monitoring and classification of *P. brassicae*-field pathotypes in OSR cultivation areas is of prime importance.
Between 2012 and 2016, 100 new clubroot-infested fields were identified in 12 German federal states. Incidence of clubroot varied within these fields from 22% to 90%. Among all *P. brassicae*-field populations, four distinct virulence patterns (P1, P2, P3 and P5) were identified according to Somé (1996). 28 isolates were able to overcome the resistance of clubroot-resistant OSR cv. Mendel. The investigation on soil samples confirmed that clubroot appears over a wide range of pH, from 5.1 to 8.3, but that a significant negative correlation occurs between disease incidence and soil pH. Furthermore, more cases of disease and severe incidences were observed in sandy loam and loamy sand as compared with other soil types.
In parallel field studies, resistant cultivars and soil amendment with calcium cyanamide and burnt lime alone or in different combinations applied before or after sowing were examined for the potential strategies for suppressing the clubroot disease. Soil moisture, soil temperature and soil pH at two different depths (15 and 30 cm) were measured at regular intervals over the growing season. Clubroot disease incidence and severity were assessed visually for the development of root galls. Field results showed clear differences between the treatments. Resistant cultivars remained the more effective management strategy providing 60-80% disease control. Soil amendments gave variable control between three field locations and years. Changing the time of application had a significant effect on the final severity of the disease. In comparison with calcium cyanamide, burnt lime application has a smaller effect.

Keywords: *Brassica napus*, pathotype, disease severity index, soil amendment, soil pH, calcium cyanamide

Session 2
Poster 13

Phenotypic variability between some isolates of *Botrytis cinerea* and *Botrytis pseudocinerea* collected in the region of Bejaia (Northern Algeria)

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Botrytis cinerea is an ubiquitous pathogen which causes severe losses in many fruit, vegetable and ornamental crops. The pathogen infects leaves, stems, flowers and fruits. In the present study, eight single-spore strains of *B. cinerea* isolated from tomato greenhouses located in Bejaia regions (Northern Algeria). Isolates were molecularly characterized by nine microsatellite makers. Isolates were assigned as *B. cinerea* and *B. pseudocinerea* with four isolates of each species. Morphological characterization was established using two cultures media (PDA and MEA). All isolates inoculated on PDA medium were exclusively Sclerotial, in contrast all isolates were Mycelial on MEA medium. The strains of the two species were similar in aggressiveness on both host species tested (tomato leaves and apple fruits). Moreover *B. cinerea* isolates were more aggressive than *B. pseudocinerea* on lettuce leaves. Both plants (tomato leaves and lettuce leaves) were significantly more susceptible to B. cinerea and *B. pseudocinerea* than those of apple fruits. A significant negative correlation was established between aggressiveness and morphological type. The epidemiological consequences concerning populations of *B. cinerea* and *B. pseudocinerea* in tomato greenhouses are discussed.

Keywords: *Botrytis pseudocinerea*, *Botrytis cinerea*, Morphology, Agressiveness

Session 2
Poster 14

Detection of *Sclerotinia sclerotiorum* in oilseed rape using real-time PCR

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Sclerotinia sclerotiorum is a plant pathogen causing Sclerotinia stem rot, which is a major disease in oilseed rape in Sweden. The pathogen can survive in the soil as sclerotia for at least ten years. Four field trials were performed in Örebro County, Sweden, during the years 2012 and 2014-2016 in order to study the occurrence of the pathogen and the disease. Real-time PCR (qPCR) was used to quantitatively detect *S. sclerotiorum* in oilseed rape leaves as well as in air samples. The leaves of the oilseed rape were collected at BCCH 63 and air samples were collected using a Burkard spore trap. The field experiments conducted 2012 and 2014 were performed in spring oilseed rape and showed a low to high stem rot incidence for year 2012 (maximum 24%) and very low incidence for 2014 (0-2%). The results from the qPCR showed a variation in incidence of *S. sclerotioum* DNA on the spring oilseed rape leaves from 10 to 100%. During 2015 and 2016, the field experiments were performed in winter oilseed rape and stem rot was detected in all fields. In five out of ten and nine out of ten winter oilseed rape fields the incidence of stem rot was equal to or above 15%, respectively. The highest infection level, 62%, was assessed in a field in 2016. Leaves from winter oilseed rape are still being analyzed. The results from the spore trap showed that *S. sclerotioum* DNA can be detected in the air from the beginning of May until the beginning of August. The obtained qPCR data in combination with stem rot incidence and climate data are being employed in the development of a computational prediction model that could potentially be used to improve disease risk assessment.

Keywords: *Sclerotinia sclerotiorum*, real-time PCR, oilseed rape, spore trap, prediction model

Session 2
Poster 15

The EF1α region as a target to assess *Fusarium* diversity on cereals using a high-throughput sequencing technology

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Fusarium head blight is a major cereal disease caused by a complex of *Fusarium* species. These species vary in importance depending on climatic conditions, agronomic factors or host genotype. In addition, most of the *Fusarium* species are able to produce one or more mycotoxins with varying degrees of toxicity in the grain. These mycotoxins constitute an important food safety concern, as they have health implications to both humans and animals. *Fusarium* species involved in FHB differ in their pathogenicity, ability to produce mycotoxins and fungicide sensitivity. Accurate and exhaustive identification of *Fusarium* species *in planta* is therefore of great importance.
In this study, a new set of primers targeting an informative region of the EF1α gene was developed. The primers were evaluated for their specificity on a panel of DNA extracts representing 30 *Fusarium* species and 23 non *Fusarium* species. High throughput sequencing protocol using Illumina technology and bioinformatics analyses steps were optimized and validated on mock communities and infected grain communities. *Fusarium* species could be retrieved from the mock and infected grain communities and good reproducibility could be observed according to the different parameters tested (DNA extraction, wheat cultivar, PCR cycle number, sequencing runs). One infected wheat grain could be retrieved in 10000 uninfected grains using this technology. The tool was further tested on 65 field samples of durum wheat, soft wheat and barley collected at various locations in France and up to 17 *Fusarium* species could be detected.
This new set of primers allows evaluating diversity of the *Fusarium* complex on cereals using high throughput sequencing. It provides a more exhaustive picture of the *Fusarium* community than the currently used techniques based on isolation or species-specific PCR detection. This new experimental approach may be used to show changes in the composition of the *Fusarium* complex or detect the emergence of new *Fusarium* species in response to various environmental factors which is of great concern for managing the disease and predicting mycotoxin contamination risks.

Keywords: *Fusarium*, high throughput sequencing, metabarcoding, wheat

Session 2
Poster 16

Barley soil borne mosaic viruses: Identification of predominant viruses affecting yield and malting quality, in order to orientate breeding towards a sustainable resistance

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Genetic resistance is the only way to control mosaic damage in winter barley. At least 18 resistance genes are known, but until recently, rym4 was the most used by breeders to control *Barley yellow mosaic virus*-1 (BaYMV-1), predominant in France and *Barley mild mosaic virus* (BaMMV). However, since 2009, new significant damage has been observed in malting barley in a widening production area, indicating possible overcoming of resistance, with the development of BaYMV-2. The objectives of the Mosa-hordeum project were to: identify new viruses or pathotypes; develop new detection tools; confirm cultivar resistance observed in field using MB tools; confirm efficiency of resistance genes cited in literary reviews; quantify impact on yield and malting quality. A complete viral inventory was carried out 2013- 2016 on affected barley crops in 108 sites using: real-time PCR; Sanger sequencing; Next-Generation Sequencing; and serological tests. A new tool, based on derived Cleaved Amplified Polymorphic Sequences (dCAPS), developed to investigate BaYMV-1 and 2 distributions, demonstrated that BaYMV-2 is predominant (> 95%) in diseased samples. BaYMV-1 and BaMMV were also identified in co-infection with BaYMV-2 on susceptible cultivars. Comparison of yield components, malting and beer quality obtained on healthy/contaminated plots with couples of cultivars revealed variation in yield losses, up to 3t/ha, reduction in number of spikes and kernels/m², smaller kernels, slight increase in protein content and decrease of malt extract. Phylogenetic analyses indicated that the *rym4* resistance-breaking ability of BaYMV-2 independently evolved on multiple occasions. In limited number of trials, *rym5* resistance was overcome by a variant of BaMMV. The implantation of differentials in 21 contaminated trials confirmed the efficiency of 11 resistance genes against the BaYMV-2/BaMMV complex. To monitor BaYMV and BaMMV resistance for registration in the National List real-time PCR appears more efficient than dCAPS. These findings will help breeders achieve sustainable resistance.

Keywords: BaYMV 1 & 2 detection, resistance genes, yield, malting quality, barley

Session 2
Poster 17

Spatiotemporal dynamics of inoculums of Sigatoka disease of banana at plot and plant scales

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Sigatoka disease (SD), caused by the fungal pathogen *Mycosphaerella musicola*, is a very destructive foliar disease of bananas spread by conidia and ascospores. A good understanding of epidemics progress at different spatial scales is essential to improve present control practices.
Our aim is to describe and to quantify the spatiotemporal dynamics of the primary and secondary inoculums at plot and plant scales on three banana cycles.
In Guadeloupe, 800 vitroplants were planted and observed after natural infections during 30 months. The spore dispersal was described by following the incidence at plot scale and by quantifying lesions at plant scale. The primary and secondary inoculums were monitored mensually by using volumetric spore traps, at two heights (above and under plant approximating the both inoculum capture). The secondary inoculum was also quantified at plant scale by numerating and sporulating lesions on different leaves.
Results showed that the airborne inoculum is spatially randomly dispersed at plot scale, for the primary and secondary inoculum and for two successive banana cycles. At plant scale, lesions had the same spatial distribution on the different compartments of the leaf, and they were more numerous on the top compartments than on the bottom. The conidia concentration in the air was always under 60 conidia/m^3. This conidia concentration was twice higher in the secondary than in the primary inoculum. There was not relationship between the dynamics of the conidia in the air and the rainfall. The ascospores concentration could not be achieved. At plant scale, the secondary inoculum quantified through the average lesion number on the top compartment varied from 3 to 225/25 cm^2. The dynamics of the appearance of lesions was different between banana cycle.
These results gave new knowledge on spatial dispersal at plot and plant scale and on relative part of primary and secondary inoculums.

Keywords: Sigatoka disease, spatiotemporal dynamics, spore traps, lesions counting

Session 2
Poster 18

In vitro expression of human phage-displayed scFv to detect potato virus Y

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Potato virus Y (PVY), the most notorious plant virus, causes a very serious problem in potato. To prevent the spread of plant viruses, there are so many diagnosis and detection techniques as serological procedures like enzyme-linked immunosorbent assay (ELISA). Serological methods have been used widely with whole antibody or recombinant antibody like single-chain variable-fragment (scFv). Screening from recombinant antibody (scFv) library with phage display technology has been used for more convenient antibody production. However, there are some difficulties in obtaining or expressing specific antigens used for animal immunization or screening. To overcome this limitation, recombinant coat protein (CP) of PVY displayed on yeast surface was used as antigen in this study. Bio-panning for selection of anti-PVY scFv was performed with human scFv library against recombinant yeast antigen. The antibody-antigen binding activity of scFv candidates was measured by enzyme-linked immunosorbent assay (ELISA) test. Selected scFv was expressed as both phage-displayed and soluble scFv. The scFv-displayed phages were precipitated by using PEG/NaCl solution and used for expedite development of virus detection method. For bacterial expression of soluble scFv, the selected scFv genes were subcloned into pET26b(+) and proteins were purified using affinity chromatography. PVY-infected samples was detected by ELISA and lateral flow immunoassay (LFIA) using the anti-PVY scFv. The purified scFv proteins showed better antigen-specific binding activity and lower cross activity than commercially available antibodies. The horse radish peroxidase (HRP) was cloned and expressed with scFv as fusion protein to reduce reaction steps and times. Purified enzyme-linked scFv showed chromogenic activity with 3,3 ,5,5 -tetramethylbenzidine (TMB) substrate forming blue color. These results demonstrate that PVY-specific scFv proteins isolated from human scFv library using yeast surface display and phage display technologies provide new *in vitro* antibody production system for plant virus diagnosis.

Keywords: ELISA, scFv, Phage display, Potato virus Y, Virus detection, Yeast surface display

Session 2
Poster 19

Development of a rapid method to identify pectinolytic bacteria isolated from blackleg symptoms on potato field

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Soft rot pectinolytic bacteria induce blackleg disease on potato crop. Symptoms are caused by *Pectobacterium* and *Dickeya* genera and comprise several species or subspecies. Rapid and accurate identification is a crucial issue for potato industry. In this work, we develop a genetic characterization tool focus on fifty-three *gapA* genes of genomes publicly available. After alignment of sequences, thirty-five signature nucleotides specific of a genus, species or subspecies were found. Then, a PCR assay based on design of primers on *gapA* sequence was effected on twenty-two strains isolated from blackleg symptoms of French potato fields. Amplicons were sequenced and signature nucleotides were analyzed. A phylogeny reconstruction has confirmed the identification of each strain by comparison with *gapA* sequences of referent genomes and validate the approach. Finally, the method is useful for a rapid and accurate characterization of all soft rot pectinolytic bacteria isolated in this study: *P.atrosepticum*, *P.parmentieri*, *P.carotovorum* subsp. *brasiliense* G1, *P.carotovorum* subsp. *carotovorum* G2 and G3, *P.carotovorum* subsp. *odoriferum* G4, *D. dianthicola* and *D.solani* were all identified with the developed tool. Moreover, sequences can be stored and re-used for further analyses.

Keywords: identification, soft rot pectinolytic bacteria, blackleg disease, potato crop, phylogeny

Session 2
Poster 20

Lettuce in Belgium has fungal and nematode problems

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Lettuce is a very important crop in Belgium and is mainly produced in glasshouses with up to 5 rotations per year. In 2015 the production of lettuce generated a revenue of almost 25 million euro, with a production of 42 800 tonnes on 200 ha. As a result of this very intensive production, the crop suffers from soil-borne diseases, caused by various pathogens including *Rhizoctonia solani*, *Pythium spp*., *Botrytis cinerea* and *Sclerotinia spp*. These pathogens cause symptoms commonly described as basal rot. In addition, nematodes such as Paratylenchus spp. and Pratylenchus penetrans cause root damage resulting in reduced growth. Since the complete ban of methyl bromide in 2006, control strategies rely on intensive use of fungicides and nematicides, resulting in unwanted residues in the soil and the end product. Furthermore, the Belgian lettuce production is since 2015 threatened by lettuce wilt caused by a new race *of Fusarium oxysporum f. sp. lactucae*, for which there is currently no control method available. To make disease control in intensive lettuce production more in accordance to the current IPM guidelines, we are collaboring in a project called FUNSLA with the aim to develop a decision support system for soil-borne pathogens. We are studying the epidemiology and activity of these soil borne pathogens in detail, so that the right chemicals are used only when necessary. In addition, the potential of biocontrol agents that reduce inoculum build-up in the soil is investigated. Damage thresholds for nematodes have been established to avoid unnecessary use of nematicides. To determine the activity of the different pathogens causing basal rot in different seasons, three glasshouses are continually replanted with lettuce and monitored without any disease control. Depending on the season, several different R. solani anastomosis groups could be distinguished in a same glasshouse. In winter, we mainly isolated R. solani AGBI from lettuce crops with rotting symptoms, while in other seasons R. solani AG1-1B, AG2-1 and AG4 appear to be dominant. We are currently studying the pathogenicity and temperature range of these anastomosis groups in more detail.
Poster session

Keywords: *Rhizoctonia solani*, *Fusarium oxysporum*, nematodes, basal rot, seasonality

Session 2
Poster 21

Virulence characterization of *Podosphaera xanthii* populations in main Mediterranean melon production areas

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Melon resistance to powdery mildew, caused by *Podosphaera xanthii*, is a key component to control this major worldwide melon production constraint. Five races based on four melon lines have been described in *P. xanthii* in Europe. However, the relevance of this race system can be questioned by the fast race evolution in the last decade and the limited knowledge of the virulence diversity in populations present today in European melon production areas, impeding resistant variety development and deployment adapted to target markets.
To better understand *P. xanthii* virulence distribution in Europe and develop solid basis to redefine race nomenclature, a French Agriculture Ministry funded project has been initiated in 2012.
Through this project, a collection of more than 200 *P. xanthii* isolates has been built from samples of melon and other cucurbits collected in 2013 and 2014 in major melon production countries (France, Spain, Italy, Morocco, and Turkey). Within this collection, virulence of 125 isolates has been characterized on a panel of 30 melon lines, including the four lines defining *P. xanthii* races and 26 lines representing major powdery mildew resistance sources. Virulence was determined by detached leaf disc assay according to the variety registration protocol.
Based on the existing race nomenclature, melon isolate virulence appeared homogeneous in all regions, with 75% isolates belonging to the race 3.5. However, if considering the whole panel of 30 lines, a very complex pattern of virulence profiles has been pointed out with more than 75% of melon isolates virulent on a combination of 15 or more lines. In order to reduce this complexity, line and isolate sub-panels have been selected by statistical analysis and characterized on detached leaf disc and whole plant. Results from this analysis will be used to redefine a race nomenclature more adapted to the existing *P. xanthii* virulence situation.

Keywords: powdery mildew, melon, virulence, race nomenclature

Session 2
Poster 22

A new diagnostic multiplex polymerase chain reaction scheme for three species of *Pantoea* threatening rice production in sub-Saharan Africa

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Members of the genus *Pantoea* are responsible for many diseases of economically important crops worldwide. Emerging diseases of rice due to infection by *Pantoea* cause significant damage in most rice-growing areas in sub-Saharan Africa. The aim of this study was to develop a diagnostic multiplex polymerase chain reaction (PCR) assay for rapid, sensitive and simultaneous detection of the *Pantoea* spp. belonging to three major species of *Pantoea*, *Pantoea ananatis, Pantoea stewartii* and *Pantoea agglomerans*. Genus- and species-specific primers targeting four housekeeping genes of *Pantoea spp* were designed through a bioinformatics pipeline using multiple genome sequences. Sensitivity of detection was monitored on isolated DNA, on in vitro-grown bacterial cells, on artificially contaminated rice seeds, on artificially inoculated rice leaves and on symptomatic and asymptomatic leaves collected from affected rice fields. The reaction parameters were optimized for a multiplex PCR scheme and applied on a total of 183 *Pantoea spp* strains. The multiplex PCR scheme accurately revealed the presence of pathogens on rice seeds and leaves. This is the first report of a method allowing simultaneous detection of three important *Pantoea* spp., which will be useful in epidemiological surveillance programs.

Keywords: Multiplex Polymerase Chain Reaction, Housekeeping gene, *Pantoea spp*, rice

Session 2
Poster 23

Epidemiology and control of beet rust (*Uromyces beticola*) on sugar beet

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Increasingly high disease pressure from beet rust (*Uromyces beticola*) has been registered in recent years in Denmark. This challenge the common belief that beet rust is a minor disease on sugar beets which seldom requires control. Thus, there is a need to gain knowledge of the pathogen's biology and epidemiology including factors contributing to spread of the disease and the onset of an epidemic. As part of a project investigating IPM solutions in sugar beet, the occurrence of *U. beticola* spores in the air above sugar beet fields was monitored using Burkard 7 day recording volumetric spore traps and qPCR in four consecutive field seasons. Spores were detected throughout the sampling period and were constantly present when first disease symptoms were seen in the field. A disease cycle with all major steps was established including means of overwintering and spread. In coastal regions, sea beets can act as a green bridge and provide the primary inoculum to the sugar beet crops. Locations with early onset of beet rust were investigated and indicated a connection between early beet rust occurrence and the presence of local wild sea beets. It has been demonstrated that attacks of beet rust causes yield loss, but high disease pressure did not translate unambiguously into decreased yield or economic return in all trials. In high risk areas disease pressure can be reduced by growing less susceptible cultivars and by application of strobilurin or triazole fungicides.

Keywords: *Beta vulgaris*, spore trapping, fungicide, disease cycle

Session 2
Poster 24

Predicting abundance of *Botrytis cinerea* airborne inoculum to forecast grey mould epidemics

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Botrytis cinerea is an airborne disseminated ascomycete that causes grey mould on more than 200 species, among which some are cultivated worldwide and have a great economic importance (grapevine, lettuce, tomato). Predictive tools could help growers to rationalize their crop health management practices, particularly the timing for chemical control and thus lower the cost of crop protection and preserve the environment while maintaining the efficacy of disease control. There is a need of a forecasting system that can predict abundance of viable inoculum in the air in French agricultural areas.

We conducted a study over a 3-year period to characterize the temporal evolution of the abundance of *B. cinerea* inoculum on a non-cultivated site (region of Avignon) and to determine if climatic parameters can serve as predictive parameters in a future grey-mould risk forecast model. Local meteorological data were acquired continuously on a climatic platform. Backward trajectories of air masses arriving in Avignon on the sampling days and climatic parameters these air masses encountered along their way were computed using the software HYSPLIT. Generalized linear models with Poisson distribution were then evaluated to model *B. cinerea* abundance. Models conditional on either local climatic variables or climatic variables from backward trajectories or on both types of variables were considered. Cross validations were performed to identify the best model.

Viable inoculum was observed for 96% of the sampling days. The abundance of this viable inoculum was positively correlated with average daily relative humidity and negatively correlated with air temperature and solar radiation. The analysis of backward trajectories suggested that air masses originating from the North or the South brought more viable inoculum than those from the West. Moreover, the model providing the best prediction criterions was obtained when local climatic parameters but also, climatic parameters along the air mass trajectories and their origin were taken into account.

Keywords: aerobiology, air mass trajectory, climatic parameter, statistical model

Session 2
Poster 25

NGS and virus diagnostic: does sequence analysis strategies really matter? Results of an international proficiency testing on siRNA

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The recent developments of high-throughput sequencing (also called Next Generation Sequencing - NGS) technologies and bioinformatics have drastically changed the research on viral pathogens and is now raising a growing interest for virus diagnostics. However, any diagnostic technique has to be included in standardized protocols. Currently, a huge diversity of bioinformatics protocols for virus discovery has been reported in the scientific literature but, to date, without addressing their reliability for diagnostic purpose. The objective of this work was therefore to compare the performance of existing bioinformatics pipelines and of the result interpretation through a double-blinded large scale proficiency testing based on a set of ten fastq files and involving 21 laboratories from 16 countries. The fastq files contained 50,000 (3), 250,000 (4) and 2.5 M (3) sequences of 21-24 nt coming from 3 samples. The false positive rate was only 0.5% and mainly related to the identification of integrated sequences or misinterpretation of the results. The overall sensitivity of detection was 57 % and ranged between 35 and 100% between laboratories with a marked effect of rarefaction for some laboratories. A principal component analysis and correlation studies underlined the most important parameters for appropriate diagnostic. The repeatability of detection corresponded to 73%. This work also underlined (i) the complexity of discovering new viruses by NGS, (ii) the difficulty to detect viral pathogens with low number of siRNA reads, (iii) the inconsistencies of databases and its impact on results. Overall, this work brings key insights into the reliability of bioinformatics pipelines and underlines some key parameters for achieving a reliable detection of viruses in a diagnostic setting using siRNA sequencing.

Keywords: deep sequencing, massively parallel sequencing, bioinformatics

Session 2
Poster 26

Distribution and change in populations of *Leptosphaeria* species associated with phoma stem canker in the Czech Republic

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In the Czech Republic, the increase in area sown with oilseed rape during the last two decades has been contributing to the increased appearance of phoma stem canker. The disease is caused by two closely related fungal pathogens, *Leptosphaeria maculans* and *L. biglobosa*. The objective of this study was to assess the countrywide distribution of these two pathogens and their occurrence in oilseed rape tissues. In 2007/11, 1454 leaf spots were visually identified based on symptoms and then analysed using species-specific PCR. Out of these, 39 and 15% were detected as *L. maculans*- and *L. biglobosa*-infected, respectively, in case of single species-infected samples, while 26% corresponded to the co-infection by both species. Furthermore, some isolates, that were collected from selected leaf spots and maintained in pure cultures, were identified based on pigment production during culturing on solid and in liquid media and PCR assay. In this case, the co-infection by *L. maculans* and *L. biglobosa* in a single leaf spot appeared as well. In years 2007/12, 708 bases of oilseed rape plants divided into upper stem, base stem, root collar and tap root parts were analysed using symptom identification and PCR. The proportion of plants in which *L. biglobosa* DNA was amplified was greater than that of plants with *L. maculans* DNA and 40% of tested plants were found to be co-infected by both *L. maculans* and *L. biglobosa*. According to our results, it appears that *L. maculans* is the predominant species in autumn, while *L. biglobosa* is more successful species than *L. maculans* in colonization of oilseed rape tissues in later growth stages of a plant in conditions of the Czech Republic. This work was financially supported by MACR NAAR, projects No. QJ1310227 and QK1710397.

Keywords: phoma stem canker, oilseed rape, *Leptosphaeria maculans*, *L. biglobosa*, symptoms, PCR

Session 2
Poster 27

The ability of fungi isolated from cankers of pome fruit trees to cause fruit rots in the storage

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Apples and pears occupy an important niche in the fruit growing industry in Latvia. Emergence of fruit rot diseases and increasing damages in the orchards and storages, lack of the data on their causes and control possibilities are among the main concerns of the pome fruit growers. Several pathogenic fungi are known to cause cankers on the trees in orchards and also fruit rots in orchards and storages (e.g. *Neofabraea* spp., *Monilinia* spp.). The knowledge on these diseases is still not sufficient, and their significance in many areas is not known. The aim of the present study was to elucidate ability of various fungi isolated from tree cankers of apple and pear to cause fruit rots in the storage. Four apple and four pear cultivars differing in tolerance to fruit rots were used for the studies. Pathogenicity on fruits was characterized for 20 fungal isolates belonging to *Neofabraea* spp., *Fusarium* spp., *Diaporthe* spp., and *Valsa* spp. in two storage experiments. The differences in ability to cause fruit rot were observed among isolates belonging to the same species and among the species. Eleven of thirteen tested isolates originating from tree cankers were also able to cause fruit rot. *Neofabraea* strains and one *Fusarium* sp. isolate were most aggressive, which caused significant damages on most of the tested cultivars. The more aggressive and virulent (degree of damages and ability to infect more cultivars) were *Neofabraea* species and isolates originated from tree cankers. Tests on plants are in progress to characterize aggressiveness and virulence of these isolates on plants of the same apple and pear cultivars used in storage experiments.

Keywords: apple, pear, fruit rot, canker, *Neofabraea*

Session 2
Poster 28

Fungi and mycotoxins in annual and perennial grasses of *Leguminosae*

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The aim of study was to evaluate the contamination of growing forage plants by fungi and mycotoxins. Seventy seven samples (13 plant species of *Galega*, *Lathyrus*, *Medicago*, *Melilotus*, *Trifolium*, and *Vicia* genera) belonging to annual and perennial grasses of *Leguminosae*, collected in the different regions of the European part of Russia were analized. Comparative evaluation of the infection by *Alternaria*, *Cladosporium*, and trichotecenes producing *Fusarium* fungi in the plants samples was carried out by using real-time TaqMan PCR. The amounts of 16 mycotoxins in the plants samples were analyzed by ELISA.

The *Lathyrus* spp. in comparison with the other members of the family, characterized by high contents of the DNA of *Cladosporium* (8.7×10^{-4} ng/ng of total DNA) and *Fusarium* fungi (7.5×10^{-5} ng/ng of total DNA). The large amounts of the *Alternaria* DNA (7.8×10^{-5} ng/ng of total DNA) were found in plants of genus *Melilotus*. The perennial plants (*Galega orientalis*, *Lathyrus* spp., *Trifolium* spp.) contained more DNA of *Fusarium* fungi in compared to annual crops.

Taking into consideration *their* frequency and *concentration*, the *most* important *mycotoxins* were alternariol and cyclopiazonic acid, their highest amounts were detected in plants from genus *Trifolium*. Mycotoxins produced by *Fusarium* fungi occurred with less frequency that other mycotoxins. The greatest variety of fungal metabolites was detected in *Lathyrus* spp.

The statistical analysis showed the significant impact ($p<0.05$) of plant species on quantity of DNA of all groups of fungi and the most mycotoxins. A positive correlation between the content of the DNA of *Alternaria* and *Cladosporium* fungi ($r=+0.30$, $p<0.05$) was revealed in all samples of legumes.

In some cases the connection between amounts of the fungal DNA, mycotoxin content, the origin of samples, and the time of their collection were confirmed statistically.

Keywords: legumes, fungi, DNA, real-time PCR, mycotoxins, ELISA

Session 2
Poster 29

Use of RAPD and ISSR markers in detection of genetic variation among *Colletotrichum falcatum* Went isolates from South Gujarat India

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The present research work aims at finding genetic differences in the genomes of sugarcane red rot isolates *Colletotrichum falcatum* Went using Random Amplified Polymorphic DNA (RAPD) and interspersed simple sequence repeat (ISSR) molecular markers. Ten isolates of *C. falcatum* from different red rot infected sugarcane cultivars stalk were used in present study. The amplified bands were scored across the lanes obtained in 15 RAPD primes and 21 ISSR primes successfully. The data were analyzed using NTSYSpc 2.2 software. The results showed 80.6% and 68.07% polymorphism in RPAD and ISSR analysis respectively. Based on the RAPD analysis, ten genotypes were grouped into two major clusters at a cut-off value of 0.75. Geographically distant *C. falcatum* isolate cfGAN from south Gujarat had a level of similarity with Coimbatore isolate cf8436 presented on separate clade of bootstrapped dendrograms. First and second cluster consisted of five isolates (cfNAV, cfPAR, cfTIM, cfMAR, and cfVES) and three isolates (cfKAM, cfCHA and cfMAD) respectively, indicating the close relation among them. Twenty one ISSR primers produced 119 distinct and scorable loci in that 38 were monomorphic. The number of scorable loci for each primer varied from 2 (ISSR822) to 8 (ISSR807, ISSR823 and ISSR15) with an average of 5.66 loci per primer. Primer ISSR835 amplified the highest number of bands (57), while only 16 bands were obtained by primers ISSR822. Four primers namely ISSR830, ISSR845, ISSR4 and ISSR15 showed the highest value of percentage of polymorphism (100%). The results indicated that both of the marker systems RAPD and ISSR, individually can be effectively used in determination of genetic relationship among *C. falcatum* accessions collected from different parts of south Gujarat.

Keywords: *Colletotrichum falcatum*, ISSR, RAPD, Red Rot, sugarcane

Session 2
Poster 30

Epidemiology of Pasmo and *Septoria linicola* resistance in French flax cultivars

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Septoria Linicola is the causal agent of Pasmo, a worldwide disease of *Linum usitatissimum* (flax, i.e. 'linen' and 'linseeds' used for fibers and food oil, respectively). While severe outbreaks have occurred in France in last years, biotic and abiotic factors favoring epidemics are little known and host resistance level is still undetermined. A 3-year survey of Pasmo was performed in order to characterize the sources of primary inoculum, the early stages of epidemic, and host resistance level for some of the most deployed French cultivars.

Cohorts of *S. Linicola* fruiting bodies were observed and accurately described (type, number, maturity) on linseed stubbles during fall and winter and disease was assessed in flax fields. Pseudothecia, asci and ascospores of *Mycosphaerella linicola*, the sexual form of *S. linicola*, were identified for the first time in France on linseed stubbles. Pseudothecia appeared late summer, with a peak in October. The temporal dynamic was notably similar for the three years, with however a significant shift depending on the climatic conditions of early autumn (dryness). Ascospores are probably responsible for primary infection on winter linseed, while pycnidiospores contribute mainly to the epidemic development on spring linen and spring linseed. Finally, we conclude that both types of spores are involved in French conditions; their relative importance probably depends on the type of crop, the epidemic stage, and the climatic conditions.

Under controlled conditions, 22 varieties (15 linen and 7 linseed) were examined for Pasmo attacks on cotyledons, leaves and stems using 6 *S. Linicola* isolates. The range of susceptibility was relatively wide and no significant, high level of resistance was detected. Nevertheless, linen appeared significantly more susceptible than linseed.

These results help to understand how Pasmo develops and to identify management options adapted to the diversity of flax cropping practices in France.

Keywords: flax, host resistance, linen, linseed, *Linum usitatissimum, Mycosphaerella linicola*, Pasmo, primary inoculum, *Septoria linicola*, sexual reproduction

Session 2
Poster 31

Detection, identification and quantitation of oomycetes in imported nursery plants

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International trade in hardy ornamental nursery stock, including Internet sales, has increased the dispersal of many plant pathogens, including highly damaging oomycetes. Potted plants with soil substrates are now recognised as the major pathway of introduction of new pests and pathogens into Europe. We screened the diversity and pathogen loads in soil from potted woody plants, in roots and in water using baiting assays. Classical isolation techniques were used as well as molecular assays, including TaqMan PCR chemistry and Next Generation Sequencing approaches using a multi-locus Illumina MiSeq. Approximately 90% of tested plants contained at least one species of oomycete (Phytophthora, Pythium, Phytopythium), while 86% of asymptomatic plants tested positive for oomycetes in the growth substrate. Ten Phytophthora species, 17 Pythium spp. and 5 Phytopythium spp. Were isolated using classical techniques. TaqMan assays for three different loci (ITS, trnM-trnP trnM and atp9-nad9), two of them Phytophthora spp. specific, revealed higher pathogen average densities in soils in comparison with the root and filter samples. The multi-locus Illumina assay demonstrated the high diversity of oomycetes present in all samples analysed, including asymptomatic plants, revealing high abundance of potentially harmful species such as Phytophthora ramorum and P. alni. These results provide worrying evidence of plant pathogens being moved 'silently' between countries in nursery plants, and highlight the need for stronger regulation to reduce plant biosecurity risks in Europe.

Keywords: oomycetes, multi-locus, TaqMan, Illumina MiSeq, ornamental plants, international plant trade, Internet sales

Session 2
Poster 32

Occurrence of *Plasmodiophora brassicae* Wor. and virus diseases of oilseed rape (*Brassica napus* subs. *napus*) in the Czech Republic

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Clubroot, caused by *Plasmodiophora brassicae* (Wor.), has been spreading on winter oilseed rape (*Brassica napus* L.) in the Czech Republic over the past six years. Clubroot infestation and spread were monitored over five years and maps of infestation were created. Experiments with clubroot resistant cultivars of winter oilseed rape were carried out in the field and greenhouse. In the greenhouse, six resistant cultivars were grown in infested soil collected from various fields in the Czech Republic, and assessed for disease severity. The soil samples were also tested for the presence and amount of *P. brassicae* inoculum by conventional and quantitative PCR analysis. In the field experiment, seven resistant cultivars were grown and disease development was monitored monthly. Yields were measured at the end of the cropping season. Finally, a set of 17 *P. brassicae* field isolates from across the Czech Republic were assessed for pathotype designation on the differential hosts of Williams, Somé et al., and the European Clubroot Differential set. The information obtained on the effectiveness of host resistance and pathogenic diversity of *P. brassicae* populations from the Czech Republic may help to more effectively manage clubroot in this country.
In autumn 2016 the unusually high abundance of green peach aphid (Myzus persicae) occurred on oilseed rape fields across the Czech Republic. This species is a vector of *Turnip yellows virus* (TuYV) and *Turnip mosaic virus* (TuMV), which are commonly found on oilseed rape. The nationwide monitoring of these two viruses was made using detection by Triple Antibody Sandwich Enzyme-Linked ImmunoSorbent Assay (TAS-ELISA) and Double Antibody Sandwich ELISA (DAS-ELISA). The test revealed high occurrence of TuYV - 93.7 % of tested samples were positive. On the other hand, the occurrence of TuMV was very low – just 0,2 % of samples were positive. The spring monitoring of virus occurrence is planed as well as testing of oilseed rape cultivars, which are declared as resistant.

Keywords: clubroot-resistant cultivars, pathotypes, qPCR, Myzus persicae, *Turnip yellow virus* (TuYV), *Turnip mosaic virus* (TuMV)

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Poster 33

Eruca sativa naturally infected by *Turnip mosaic virus* in Brazil

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Leaves of rocket salad (*Eruca sativa* Miller) with mosaic symptoms were observed in a commercial crop production and investigated for the presence of viruses. Associated to these plants we also found raphanus (*Raphanus raphanistrum* L.) with mosaic symptoms. Filamentous particles typically of potyvirus were observed by Electron Microscopy and the plants were positive for ELISA Test using the potyvirus antiserum (Agdia, Inc). Total RNA was extracted by Bertheau et al., (1998) followed by RT-PCR using the universal primers for potyvirus (W-CIEN and PV-1, described by Gibbs & Mackenzie, 1997). The amplified fragment showed 99% identity with *Turnip mosaic virus* (TuMV, accession number EU734433.1). A couple of primers were synthesized to amplify the complete coat protein region (forward: 5' ACAGATGAGCAGAAACAGGC 3' and reverse: 5' AATCAAATGCGTACCGAGC 3') and identity of 97% was observed with TuMV (accession number AB701725.1). Bayesian analysis using different reference sequences of TuMV isolates was performed and this isolate was grouped in the Brassica-Raphanus (BR) clade according to Nguyen et al., (2013). The virus was sap transmitted to *Chenopodium amaranticolor*, *Chenopodium quinoa* that developed local lesions, *Beta vulgaris* (local lesions and systemic necrosis), *Nicotiana benthamiana* (mosaic symptoms), to rocket and raphanus plants that developed local lesions followed by mosaic and necrosis of leaves. The isolate of TuMV was also efficiently transmitted to two rocket genotypes (astro and rococó) and raphanus plants by *Aphis gossypii.* As far as we known, this is the first report of rocket salad infected with TuMV in Brazil.

Keywords: Potyvirus, rocket salad

Session 2
Poster 34

Characterization of genetic diversity of *Venturia inaequalis* population in Latvia using microsatellites

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Apple scab, caused by the heterothallic ascomycete *Venturia inaequalis* (Cooke) G. Wint. is one of the most important diseases of apple worldwide. Due to the life cycle of *V. inaequalis*, in each spring apple trees are infected with newly released ascospores representing new genotypes of the pathogen. This phenomenon has been reported to have one of the major impacts on diversity and formation of more aggressive races of *V. inaequalis*. Set of 99 single spore isolates originated from different regions, orchards and cultivars were genotyped with 14 polymorphic microsatellites (SSR) markers to characterize genetic diversity of *V. inaequalis* population in Latvia. The set of isolates also included several strains from Belgium, France, Poland, Germany, Netherlands obtained from culture collections for comparisons. Resulting binary data set was analysed by cluster analyses UPGMA, Neighbour Joining and principal component analysis (PCA). In UPGMA analyses Latvian origin isolates clustered according to samples, trees and partly also to orchards. In PCA analysis several separate groups were identified among Latvian origin isolates according to the region, orchard, and cultivar. Some of the Latvian *V. inaequalis* isolates showed genetic similarity to isolates virulent on scab resistant cultivars from other European countries indicating the potential for further virulence development.

Keywords: Apple scab, SSR markers, fungal diseases, *Malus domestica*

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Poster 35

Comparison of crown rust resistance reaction of oat differential lines obtained from different sources

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Rusts are known as the most widespread, destructive and economically important diseases of cereal crops worldwide. Necessary information about fluctuations in the racial composition, race prevalence, arising of new races and effectiveness of resistance genes can be obtained by rust population changes monitoring. Information provided are especially important to breeding programs in order to decide on, what breeding strategy for resistance should be taken, which genotypes should be cultivated and which genes should be combined in crossing. At the heart of reliable measurements are reference materials. They give a benchmark for laboratories throughout the world to deliver accurate and comparable results of virulence surveys and molecular characterization of major cereal rusts.

This study compares crown rust resistance reaction of oat differential lines containing genes: *Pc39, Pc40, Pc46 Pc48, Pc50, Pc51, Pc58, Pc59, Pc94* and *Pc96* obtained from Iowa State University in USA and Cereal Research Centre AAFC Winnipeg in Canada by means of host-pathogen test. Assessment was conducted on ten-days-old 3-cm-long leaves fragments in the laboratory tests under controlled temperature, humidity and lighting conditions. For inoculation 378 *P. coronata* f. sp. *avenae* isolates collected in Poland during the years 2013-2016 from random farm fields and field plots were used. The results indicate that majority of the tested lines showed a homogeneous reaction with the exception of lines possessing *Pc50* and *Pc51* genes. In the isoline Pc50 on the basis of segregation in resistant and susceptible plants two lines, each possessing a different major gene *Pc50-2* and *Pc50-4* were already distinguished by Šebesta (1983). Both *Pc*51 lines in Polish conditions showed high efficiency, however when resistance was overcome, plant reaction differed. These results may indicate that *A. sterilis* Wahl No. 8, the donor of *Pc*51 line resistance could also have two major resistance genes, which was randomly reselected in later isoline generations.

Due to pathogen evolution constant rust research should be conducted in order to support disease resistance breeding as well as study coevolution of natural host pathogen systems. It is essential for researchers around the world to work with a uniform material to obtain accurately and unambiguously comparable results.

Keywords: crown rust, *Puccinia coronata*, oat, differential lines

Session 2
Poster 36

The influence of soil tillage and crop management in the agroecosystems on soil fungistasis against *Fusarium graminearum.*

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Recently a rapid spread of *Fusarium graminearum*, the main causal agent of Fusarium head blight (FHB) of cereals, has been observed in Northern Europe. The pathogen drift to the North is most likely promoted by changes in climate and farming practices. Increase of minimum tillage, continuous cropping of cereals or extremely poor rotations in many regions, along with warming climate, enables the manifestation of FHB on epidemic scale. The primary source of FHB infection is host plant residues remaining in soil. Therefore the establishment of *F. graminearum* in different agroecosystems may strongly depend on the soil capacity to suppress pathogen development and survival. The aim of this study was to evaluate the influence of different soil tillage and crop management systems on soil fungistasis against *F. graminearum*.
Soil samples were collected three times during the plant growth season, from six long-term cereal based crop rotation field trials and from a long-term experiment set up under conventional tillage, reduced tillage and no-tillage management. Soil fungistasis was evaluated in terms of reduction of radial growth of *F. graminearum* in *in vitro* assay and expressed in percent.
Differences in soil fungistasis, among treatments investigated, in most cases were statistically insignificant. *F. graminearum* growth on unfumigated soil was reduced by 80% compared to chloroform fumigated soil in the tillage management experiment and by 70% - in the fields of different crop rotations.

Keywords: crop rotation, fungistasis, soil suppresion, tillage minimisation

Session 2
Poster 37

Reaction of stem base diseases causative agents to fungicides and impact of agronomic practices to eyespot incidence on cereals

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The control practice of foot-rot diseases is mostly based on the use of fungicides. In 18-year´s monitoring we analysed the main factors influencing foot-rot diseases occurrence. The highest rate of *M. nivale* infection was found in 2006 (37.0 %) and the lowest one in 2014 (13.0 %). *Oculimacula spp*. occurrence was variable between years with mean level around 6.0 %, maximum (7.6 %) in 2008 and minimum which did not exceed 1.0 % in 2012.
Early date of sowing and wheat as preceding crop were the main factors increasing eyespot infection.
This research was supported by Ministry of Agriculture of the Czech Republic, projects No. QJ1530373, QJ1310091 and RO0211.
The responses of both pathogens to prochloraz were assessed in period 2012 – 2016. Both *M. nivale* and *Oculimacula spp.* populations were sensitive to prochloraz with mean levels between 0.01 - 0.06 µg ml $^{-1}$ and ≤0.1 µg ml $^{-1}$respectively.

Keywords: cereals, fungicide resistance, agronomic practices, preceding crop, sowing term

Session 2
Poster 38

Plasmodiophora brassicae propagates at different rate in Winter Oilseed Rape crop rotations

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Clubroot caused by the soil-borne obligate endoparasite *Plasmodiophora brassicae* is now considered as the most serious disease in Oilseed Rape production in several European countries, associated with appreciable yield losses. Outbreaks of clubroot are continuously reported in winter oilseed rape (WOSR) in Sweden with a sharp increase in 2016. Crop rotation is one of the most valuable tools for disease management. The objectives were to study the influence on yield of WOSR in crop rotations when WOSR was grown at different time gaps.
WOSR was grown every (a) second, (b) third and (c) sixth year respectively in three 7- year rotations alternating with winter wheat. Two field trials were established at experimental farms in south west Sweden and WOSR was grown year 1 and 7. At one experimental site WOSR was replaced by spring oilseed rape year 7. Analyses of the propagation of inoculums of *P. brassicae* were performed by qPCR.
The significant highest yield of OSR was measured in treatments with WOSR grown every sixth year and out yielded WOSR grown every second and every third year by 37 % and 18 % respecievely. *P. brassicae* was not detected in soil sampled at the onset of the study, while a large multiplication is recorded at the end of experiments for treatments a and b., while the level was low in treatment c.
The results clearly show that *P. brassicae* (as measured by fg DNA g^{-1} soil) propagates in fields with high intensity of OSR, despite inoculums were below detection level, and points at the advantage to regularly use soil analysis as a management tool in OSR production.

Keywords: WOSR, yield loss, *Plasmodiophora brasssicae*, qPCR

Session 2
Poster 39

DNA extraction and PCR amplification method suitable for herbarium-stored speciments of *Phoma*-like fungi.

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Herbarium collections are the enormously important biological resources for studies of fungal biodiversity. The aim of this study was modification of DNA extraction method for herbarium specimens of the pycnidial fungi *Phoma s.l.* for next sequencing and molecular-phylogenetic studying.
Plant samples for this study were obtained from Herbarium collection of phytopathogenic fungi at All-Russian Institute of Plant Protection (LEP). The collection year of specimens ranged from 1897 to1959.
To pulverize plant's tissue with fungal structures and glass sand, two ball-mills (Retsch MM400 and Fast Prep 24) were used at 30 Hz/s. for 10 and 6 minutes respectively.
Isolation of total cellular DNA was carried out using CTAB-based protocol with minor modifications.
The ITS region was amplified according to method of Nested-PCR using outer (NSA3/NLC2) and inner (NSL1/NLB4).
The Nested-PCR was successful for most samples, only for few samples reaction didn't provide us with any PCR-products. Successful PCR resulted in 2 bands of approximately 600-700 b. p. and 900-1000 b.p. Bands with larger size are probably non-specific product of amplification of plant's DNA. Bands with size 600-700 b. p. represent specific amplicons of fungal DNA. Particular nested-PCR and ascomycete-specific primers helped to decrease amplification of plant's DNA and has resulted in preference of amplification of fungal DNA.
Sequencing of ITS-region was effective for 8 samples and has resulted in 600-700 b. p. fragments. With help of BLAST-tool with obtained data, 4 samples were defined to species level (*Chaetopyrena pennicillata, Leptosphaeria maculans, Boeremia exigua*), the rest 4 samples were defined to generic level (*Phoma* sp, *Boeremia* sp.). The oldest sequencing specimen was dated-by 1897 (120 years-old).
The proposed modified CTAB-method to extraction DNA from herbarium material of *Phoma*-like fungi and optimized amplification protocol generally are regarded to be reliable and useful to study herbarium-stored phomoid fungi with help of molecular methods.
Presented work was support by Russian Science Foundation (project 14-26-00067).

Keywords: mycological herbarium, biodiversity, phytopathogenic fungi, pycnidial fungi, DNA extraction, *Phoma*

Session 2
Poster 40

Tomato yellow leaf curl virus detection from commercially available tomato seeds

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Tomato yellow leaf curl virus (TYLCV) is one of the most well-known tomato-infecting begomoviruses and transmitted by sweet potato whitefly *Bemisia tabaci*. Seed transmission has previously been reported for some RNA viruses, but TYLCV has not previously been described as a seed-borne virus. In 2013 and 2014, without whitefly-mediated transmission, TYLCV was detected in young tomato plants germinated from fallen fruits produced from TYLCV-infected tomato plants in the previous cultivating season. In addition, TYLCV was also detected in seeds and their young seedlings of TYLCV-infected tomato plants that were infected by both viruliferous whitefly-mediated transmission and agro-inoculation. The seed infectivity was 20-100%, respectively, and the average transmission rate to seedlings was also 84.62% and 80.77%, respectively. Sequences were identical among TYLCV genomes of inoculum and those isolated from seeds and their seedlings. When tomato plants germinated from TYLCV-infected seeds (as donor plants), non-viruliferous whiteflies (as insect vectors) and healthy tomato plants (as receiver plants) were placed in a whitefly-free insect cage together. TYLCV was detected from whiteflies as well as receiver tomato plants six weeks later. Infectivity tests for TYLCV were also performed using 250 kinds of commercially available tomato seeds on sale at oversea markets from 24 countries in Asia, Africa, America, Europe and Oceania. Among them, TYLCV was detected from 38 kinds of seeds by PCR, and TYLCV genome sequences were confirmed from their amplicons. For the dissemination test, each seed was planted in an insect-free condition. Seedlings from 9 cultivars were identified as TYLCV-infected (showing 8-100% dissemination rates). Whitefly-mediated TYLCV transmission from tomato plants germinated from TYLCV-infected commercial seeds to other healthy tomato plants was also confirmed. Taken together, TYLCV can be transmitted via seeds and tomato plants germinated from TYLCV-infected seeds can be served as an inoculum source of TYLCV (or as a TYLCV reservoir).

Keywords: Begomovirus, geminivirus, reservoir, seed transmission, *Tomato yellow leaf curl virus*, whitefly

Session 2
Poster 41

Pathotyping *Melon Necrotic Spot Virus* (MNSV) in melon

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The *Carmovirus Melon Necrotic Spot Virus* (MNSV) is seriously affecting melon greenhouse production in Spain. Since the early 2000s, recessive resistance gene *nsv* has been deployed in melon commercial varieties and MNSV resistance declaration has been included in European variety registration process for melon.
Following resistance deployment, only few resistance breaking MSNV isolates have been isolated and characterized. However, in the last few years, an increasing numbers of MNSV symptoms has been reported in melon commercial fields grown with MSNV resistant varieties in Almeria region, suggesting the spreading of resistance breaking MNSV. This resistance overcoming has had a direct impact on MNSV resistance claim in commercial varieties, and emphasizes the need to define pathotypes for this virus.
In order to confirm the overcoming of the resistance conferred by *nsv* gene, MNSV isolates have been isolated from MNSV typical symptoms collected on commercial resistant varieties in Almeria region from 2011 to 2013. Virulence of six representative isolates has been compared to that of the official reference isolate, on a panel including both the resistant (VIRGOS) and susceptible (VEDRANTAIS) controls used in the variety registration process, and seven commercial varieties carrying the *nsv* resistance gene. This characterization has been carried out through an inter-laboratory ring-test and according to the official protocol used for variety registration.
All six MNSV isolates expressed typical systemic symptoms on all tested melon material, including resistant control and the seven resistant commercial varieties, whereas the official MNSV isolate could infect only the susceptible control. These results confirm virulence of some MSNV isolates on *nsv* resistance gene. It is then proposed to define as pathotype 0 isolates avirulent to *nsv* gene (in VIRGOS) and pathotype 1 isolates virulent to *nsv* gene. This MNSV pathotype classification will support a more transparent claim of resistance carried by commercial varieties.

Keywords: MNSV, melon, resistance, pathotype

Session 2
Poster 42

Development of a resistance test of squash to *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV) and *Watermelon mosaic virus* (WMV)

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Three viruses transmitted by aphids (no persistent transmission) are responsible of strong yield losses on squash crops in France and around the world: *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV) and *Watermelon mosaic virus* (WMV). For each virus, different biologic, serologic and molecular groups were described. The resistance is polygenic for CMV, ZYMV and with a major gene for WMV but always with an intermediate resistance.

The evaluation of squash resistance to viruses and the assessment of symptoms is complex due to the partial and not complete resistance and the quantitative results observed. It is important to describe the different levels of virulence of virus strains and to identify the different levels of resistance of squash varieties. There are currently no harmonized protocols to define the levels of partial resistance of squash to these viruses.

The aim of this project, in collaboration between GEVES, INRA and breeding companies (HMClause, Gautier, Monsanto, Rijk Zwaan, Sakata, Syngenta) was to acquire a better knowledge of levels of squash resistance and to define robust resistance tests to viruses. Based on the INRA's characterized collection of strains of viruses, three strains per virus (with different virulences and from different groups) were selected. These strains and different protocols from INRA and partners (stage of inoculation, optimal conditions of test, notation scale) were compared, on a panel made up of commercial varieties with different levels of resistance, to define for each virus a robust resistance test.

One strain, allowing to differentiate the different levels of resistance of varieties, was selected per virus. For each virus, a resistance test and reference materials (strain and controls) were validated. The reference collection of GEVES was characterized for the three viruses. These new protocols, used for CTPS registration, will be proposed to CPVO and UPOV for a harmonized used.

Keywords: squash, viruses, partial resistance, protocol

Session 2
Poster 43

CORKYRES: Development of a resistance test of tomato and root-stock to *Pyrenochaeta lycopersici*

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Pyrenochaeta lycopersici is the causal agent of corky-root, an important disease on tomato and root-stocks which causes brown corky lesions on roots and is responsible of yield losses (from 40% to 70% in case of strong infection). The control of corky-root is mainly based on the use of resistant varieties. The resistance recessive gene py-1 was introduced in tomato (*L. esculentum*) from *Lycopersicum peruvianum*.

A protocol of evaluation of resistance to corky-root is present in the UPOV guidelines and in the CPVO technical protocols. But the described protocols rely on a sowing or transplantation in contaminated soil and notation at flowering or fruits maturity stage. So this test is long and difficult to perform. Moreover the isolates and controls of this protocol have not been validated.

The aim of this project, in collaboration between GEVES and six breeding companies (HM-Clause, Gautier, Rijk Zwaan, Sakata, Syngenta, Vilmorin) was to acquire a better knowledge of this pathogen and to define a robust resistance test. A strain collection was established from literature and field isolates. Each strain was characterized for its morphology (appearance, growth), its physiology (adaptation to temperatures) and genotype. A pathogenicity test was developed to define the level of virulence of each strain and to select reference isolate. Different inoculums, conditions of tests, stages of inoculation and notation scales were compared to define a resistance test on tomato and root-stocks controls and on a panel, defined by the steering committee, of commercial varieties with different levels of resistance.

A collection of 16 characterized strains was made up and conservation conditions were defined. A resistance test and reference material (strain and controls) were validated. This new protocol was accepted as a new characteristic by CTPS for registration of varieties and will be proposed to CPVO and UPOV.

Keywords: tomato, root-stock, resistance, corky root

Session 2
Poster 44

Spore trap and molecular detection of fungi

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Several plant pathogenic fungi are dispersed by wind. The aim of this project was to create a basic understanding of the fungal communities present in air in agricultural and forest landscape throughout the growing seasons. This was done by collection of spore trap catches in the four main plant production areas of southern Sweden. The spore samples were collected using two types of spore traps, one active trap represented by the ionic suction trap and one passive funnel trap.
DNA was extracted from each sample and the fungal community was studied based on the ITS region by applying both Illumina MiSeq and PacBio RS II sequencing techniques. The fungal communities structures were investigated and the detected operational taxonomic units (OTUs) assigned to a species where possible. The list of species detected was compared to reports of disease development in the field crops in the surroundings of the respective spore traps.
The results form this study could be used to develop a prediction system, which potentially could be an important tool in disease forecasting and risk monitoring. The timing of the detection of the plant pathogenic fungi in the traps depends on the biology of the fungus. As an example, rust fungi (*Puccinia* sp.) were first found in the elevated traps, and later at canopy leave, which may be explained by dispersal by long distance dispersal. Spores of ergot (*Claviceps purpurea*) was first found at the canopy level and later in the elevated traps, which may be explained by the origin of these spores. *C. purpurea* sclerotia in the soil germinate via perithecia and air-borne ascospores. We expect that results form this project will be useful for development of disease monitoring and risk management of plant diseases in the future.

Keywords: ITS region, fungal community

Session 2
Poster 45

Describing the commercial life cycle of bread wheat varieties to study the influence of yellow rust epidemics in France

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Yellow rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most important diseases of bread wheat in France, especially known to have led to the rapid withdrawal from the market of susceptible varieties during the last decades. However, no study has so far examined how pathogen pressures could impact the adoption of varieties.
In this study, we proposed a set of indicators of the commercial life cycle of a variety, and we emphasized their relevance to distinguish between susceptible varieties and varieties presenting a durable resistance in several French agricultural regions over the last decades. We focused on the successive periods characterized by yellow rust epidemics, identified homogeneous agricultural regions in terms of level of yellow rust pressure, *Pst* pathotypes and landscape varietal composition, considering varieties sown on at least 10,000 ha. We applied principal component analyses and clustering methods on a set of non-redundant indicators of the commercial life cycle of varieties.
We first considered the epidemic period 1987-1991 characterized by the breakdown of *Yr6* and *Yr9* race-specific resistance genes in France. In the northwest coastline area, highly affected by yellow rust pressures, we identified eight varieties with a rapid withdrawal from the market. These varieties, either susceptible before the epidemic or concerned by the breakdown of resistance genes, appeared to be substitutable by highly resistant varieties available for the same end-use over the period.
We then considered the whole period 1980-2015 with the aim of identifying varieties characterized by durable resistance against yellow rust by cross-checking information on the commercial life cycle with information on postulation of resistance genes, changes in adult-plant field resistance level and pedigrees.
Following this procedure, a promising perspective for breeders could be a list of varieties with durable resistance promoting the long-term durability of yellow rust control.

Keywords: *Puccinia striiformis* f. sp. *tritici*, *Triticum aestivum*, race-specific resistance gene, durable resistance, yellow (stripe) rust

Session 2
Poster 46

Diversity analysis of *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) in Mali

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Understanding the diversity of pathogen populations in a regional scale is crucial in order to develop strategies for sustainable control of plant diseases. Our work deals with the study of the plant pathogenic bacterium *Xanthomonas axonopodis* pv. *manihotis* (*Xam*), which is the causal agent of cassava bacterial blight (CBB). CBB occurs in the tropics including in West-Africa where it has recently been reported in Burkina Faso and Ivory Coast, sometimes imposing severe yield losses to cassava producers. The status of CBB in Mali still remains unstudied.
To fill this gap, preliminary surveys were conducted in Dec. 2015 in two cassava production regions. Nearly 80 leaf samples were processed, leading to the isolation of about 50 *Xam* candidate isolates. Of these, 40 strains of *Xanthomonas axonopodis* pv. *manihotis* were validated through molecular diagnostic tests and pathogenicity assays. This study represents the first demonstration for the presence of CBB in Mali and enabled to initiate the establishment of a first collection of national *Xam* strains. Secondly, a MLVA strategy (Multiple-Locus Variable number tandem repeat Analysis) was developed based on the analysis of 14 microsatellites (VNTR) in order to assess the genetic diversity of the 40 strains isolated from our two regions under study, Bamako and Segou, which are 250km distant.
Our results demonstrate a greater diversity in populations of the Bamako region relative to that of Segou, with reference to the values of diversity indices. Also, comparison of all strains of Mali with a collection of 215 strains from border regions in Burkina Faso suggested that the Malian strains are less diversified. Further surveys covering most areas of cassava production in Mali are in progress to better understand *Xam* invasion routes in this country.

Keywords: Cassava, *Xanthomonas*, cassava bacterial blight, Mali, molecular diagnostic, MLVA-14

Session 2
Poster 47

Pantoea ananatis causing grain discoloration is widely distributed in the rice-growing fields of the republic of Korea

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Keywords: *Pantoea* spp., grain discoloration, *P. ananantis*, distributuition, phylogenetic analysis

Session 2
Poster 48

Influence of maize genotypes on *Fusarium* species frequencies and grain mycotoxin contamination in diverse localities of Poland

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Maize has become one of the essential crops for Poland. It has several advantages compared to commonly used wheat, mainly in terms of yield and diverse use for foods and feeds. Like all other cereal crops, it suffers from a range of fungal diseases and *Fusarium* species are among the most damaging pathogens, producing a plethora of toxic secondary metabolites, accumulated in maize grain and, thus, introduced into the food chain. Humidity and temperature are the factors determining the disease severity but geographical conditions, plant genotype and local pathogen populations play also important roles.

We analysed *Fusarium* populations infecting several maize genotypes harvested in 2015 season under the conditions of eleven localities in Poland. Grains from infected cobs were plated on water agar medium and all fungal strains were recovered. Over 400 *Fusarium* isolates were recorded and identified using molecular techniques (*tef*-1α sequence analysis and species specific SCAR markers). Consequently, grain contamination with typical mycotoxins was evaluated using liquid chromatography methods.

Species frequencies depended strongly on maize genotype (earliness group), geographical location and weather conditions. *Fusarium verticillioides* was the most abundant species in all localities, followed by *F. proliferatum* (47% and 30,5% of all *Fusarium* isolates, respectively). A number of minor species was also present: *F. temperatum* (6,3%), *F. graminearum* (5,8%), *F. poae* (5,6%), *F. subglutinans* (1,9%), *F. sporotrichioides* (1,5%), *F. equiseti* (0,5%), *F. oxysporum* (0,5%), *F. culmorum* (0,2%) and *F. tricinctum* (0,2%).

In all maize samples *Fusarium* mycotoxins (fumonisins, deoxynivalenol, zearalenone) were analysed – after extraction and purification – using chromatographic techniques (HPLC/PDA/FLD).

Our analyses have shown local variability of *Fusarium* communities in relation to host genotypes and weather conditions. Concentration levels of analysed mycotoxins were also varied and depended on location and maize genotype.

The part of this research was financially supported from the Polish National Science Centre project 2014/15/B/NZ9/02169

Keywords: deoxynivalenol, fumonisins, *Fusarium*, maize, zearalenone

Session 2
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One-step multiplex reverse transcription-polymerase chain reaction for the simultaneous detection for barley virus diseases

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Keywords: BaYMV, BaMMV, BYDV, Simultaneous detection

Session 2
Poster 50

Investigating the *Alternaria* disease progression and species composition on potato in Belgium

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Alternaria species are a serious threat for potato cultivation since heavy infections can lead to significant yield and quality losses. The infection causes necrotic symptoms, which cannot be visually distinguished. Over the past years, *Alternaria* on potato has become increasingly important in NW Europe. Although the exact cause for this emerging problem remains elusive, it might be attributed to the combined effect of climate change, a reduced use of the fungicide mancozeb, the increased specificity of active ingredients to control *Phytophtora infestans* and the production of high-yielding susceptible cultivars. Furthermore, little is known about the Belgian *Alternaria* population composition and the contribution of both *A. solani* and *A. alternata* to the disease. The main goal of this research is to identify the primary causal agents of *Alternaria* disease on potato, to determine inter- and intraspecific diversity within the *Alternaria* population in Flanders, and to unravel the complex interaction between stress-related hormones and the *Alternaria* infection. To achieve these objectives, 22 locations were monitored throughout Flanders during the growing seasons of 2014 and 2015. Results of this disease survey unequivocally show that the disease incidence and –pressure for both seasons was low and that crops grown in sandy soils appear to be more prone to *Alternaria* disease. In a second part, we identified the population composition at the species level on different time points during the growing season. Therefore, infected leaf samples were collected from the field and using a microscopic and molecular approach, we concluded not only that *A. arborescrens* is the most abundant species present on infected leafs, but also that small-spored species like *A. alternata* and *A. arborescens* rather than the large-spored *A. solani*, were predominant at the beginning of the growing season. The disease escalated only when *A. solani* species were accumulating. Indeed, based on high-throughput virulence assays, we observed that *A. solani* was much more virulent than *A. alternata* or *A. arborescens*. Next, a subset of isolates will be used to investigate the complex interaction between host-specific toxins and stress-related hormones such as ethylene or auxins during the infection process. Indeed, previous research shows that ethylene is a key component in upstream signaling of Programmed Cell Death induced by host-specific AAL-toxin in tomato.

Session 2
Poster 51

Population structure and host specialization in *Botrytis cinerea*

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The *Botrytis* genus encompasses more than 30 species, most of them being able to infect a restricted range of host plants. Nevertheless, *B. cinerea* is considered a generalist pathogen, recently found to infect 586 plant genera. This makes *B. cinerea* an interesting candidate to study ecological specialization. This was first approached while describing population structure in France, from strains collected on greenhouse tomato, grapevine, blackberry, strawberry and hydrangea. Understanding the causes of population subdivision is of fundamental importance, as studying barriers to gene flow between populations may reveal key aspects of the process of adaptive divergence and, for pathogens, may help forecasting disease emergence and implementing sound management strategies. Population genetics analyses revealed a weak association between population structure and geography, but a clear differentiation according to the host plant of origin, and especially for greenhouse tomato and grapevine. Host specialization was validated by cross-inoculation experiments, carried out *in vitro* on detached leaves. Genomes from strains contrasting for their specialization on tomato, grapevine, blackberry and hydrangea were Illumina-sequenced and genomic phylogeny analysis confirmed the similarities between genomes from strains collected on the same host. Population genomics analysis used to compare variation between and within lines of strains provided promising sets of genes that may induce host specialization. In addition to coding sequences, analysis of small RNAs from strains collected either on tomato or grapevine was initiated. Preliminary sequencing data suggest that these two populations have contrasted repertories of small RNAs. Further analysis and additional genomes and small RNA sequencing are required to definitely identify the determinants of host specialization. Our findings open up new perspectives for disease control by managing *Botrytis* hosts in agricultural landscapes, and reinforcing prophylactic measures.

Keywords: *Botrytis cinerea,* adaptation, population structure, host specialization, genome sequencing, cross-inoculation test

Session 2
Poster 52

Investigation of the fitness of *Zymoseptoria tritici* in relationship with fungicide tolerance

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Zymoseptoria tritici is currently the main pathogen on wheat crops, especially in North-Western Europe, where environmental conditions are suitable for disease establishment. Disease control relies mainly on the use of fungicides and partially-resistant cultivars, but these management strategies are frequently circumvented in the field by the fungus, due to its high biological fitness degree, resulting likely from its active sexual reproduction. Although the importance of the fitness concept to perpetuate the existing fungicides and to develop sustainable protection strategies, it remains poorly understood in *Z. tritici*. The aim of the present project was thus to decipher the fitness components of *Z. tritici* in relationship with fungicide tolerance. First, a collection of 240 single-spore isolates of the pathogen were collected in 2016 from northern France and fingerprinted with microsatellite markers to select unique genotypes. One hundred haplotypes were selected and are under assessment for resistance to sterol 14α-demethylation inhibitor (DMI) and succinate dehydrogenase (SDHI) fungicides using phenotypic and molecular assays. Results would allow an update of resistance level to these fungicide families in northern France. Furthermore, fungal strains belonging to different resistance profiles will be selected and examined for their fitness components (pathogenicity level, pathogenicity determinants, sporulation rate, etc.) and competition ability, using cytological, biochemical and molecular approaches. Findings will provide valuable information about the fitness of *Z. tritici*.

Keywords: *Zymoseptoria tritici*, *Mycospaherella graminicola*, wheat, fungicide resistance, fitness degree

SESSION 3. EMERGING DISEASES AND NEW INSIGHTS IN TAXONOMY AND PHYLOGENY OF PLANT PATHOGENS IN EVOLVING GLOBAL CONDITIONS

Session 3
Poster 53

Genetic variability within *Septoria carvi* Syd. (*Ascomycota*) – a new pathogen of caraway *Carum carvi* L.

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Fungi of the genus *Septoria* (teleomorph *Mycosphaerella*) belong to the widespread pathogens of crops and wild plants in all regions of the world. The species *Septoria carvi* is especially dangerous to caraway plants. It causes septoriosis. According to literature, *S. cari, S. umbelliferarum* and *S. carvi* are recognized as the cause of septoriosis of caraway cultivated in European countries. The purpose of our study was to investigate whether *S. carvi* is a new pathogenic species of caraway or perhaps it is one of the described species colonizing other plants of the family *Apiaceae.* Identification of the studied isolates was carried out using the classical methods as well as molecular studies using PCR. DNA was isolated from own isolates of *S. carvi.* The sequence of nucleotides rDNA of our own isolates, obtained during the study of four loci, i.e. regions ITS1- ITS2, actin (Act), calmodulin (Cal) and translation elongation factor 1-alpha (EF 1-α) were compared with the sequences of reference isolates of *Septoria* species affecting other plants of the family *Apiaceae*. Phylogenetic analysis showed distinct differences of the tested isolates, which allowed to treat them *Septoria carvi* species affecting the above-ground organs of caraway *Carum carvi* L. The obtained results showed differences between the studied barcodes and indicated that translation elongation factor 1-α constituted the best primer to identify *S. carvi.* This study is the first report on the genetic characteristics of the species *S. carvi.* The obtained sequences of *S. carvi* isolates have been submitted to the GenBank.

Keywords: *Septoria,* caraway, PCR, genetic characteristic

Session 3
Poster 54

Diseases of Scots pine (*Pinus sylvestris*) needles in selected areas of northern Poland

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Scots pine (*Pinus sylvestris*) is a crutial ecological but also economic component of Polish forests. In recent years incerased pine needle mortality rates have been observed around the country. This phenomenon is a matter of great concern because it applies to large area of forest stands.
Approximately 30-year-old pine stands have been chosen for the study and the area have been examined for two years, during which symptomatic pine needles have been collected in different conditions as it is known that foliar fungi may only produce fruiting bodies during a specific season.
During the study symptoms consisting of brown discoloration of needles have been observed in all the areas studied. The cause of the symptoms has been identified as *Sphaeropsis sapinea*, and it is probable that climate extremes in recent years have contributed to the occurrence of the pathogen to great extent. Other pathogens that have been isolated include: *Lophodermium pinastri, Lophodermium seditiosum, Cyclaneusma minus, Cenangium ferruginosum, Lophodermium pinastri, Sclerophoma pythiophila, Alternaria alternata, Gremieniella abietina and many other species.*

Keywords: Scots pine, *Pinus* sylvestris, needles, disease, pathogen

Session 3
Poster 55

Pathotypes of *Plasmodiophora brassicae* in Poland

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Plasmodiophora brassicae is a protist causing clubroot, which is a damaging disease of vegetable brassicas and oilseed rape, also found on cruciferous weeds. Still little is known about the population of this pathogen and the distribution of pathotypes worldwide. Comparison between the pathotypes described in the literature are impossible due to different systems of classification. In this study pathotypes found in Poland have been studied using three most popular differential sets, described by Williams (1966), Buczacki et al. (1975) and Somé et al. (1996). Moreover, we have used cv. Mendel – the first cultivar of oilseed rape conferring the major resistance gene derived from *Brassica rapa*, which was added to the standard differential set of Somé. The number of pathotypes varied depending on the differential system and threshold used. It was found that most of the pathogen population in Poland is composed of nearly equal numbers of the pathotypes P_1 and P_3, but as much as 38% of isolates could break down the resistance present in cv. Mendel. Based on the remaining identification systems up to nine pathotypes have been found by now. Sequences of fragments ITS1-5,8S-ITS2 of 22 isolates of *P. brassicae* belonging to different pathotypes showed no sequence polymorphisms in regions of the ribosomal DNA, whole sequence of 5,8S and studied fragments of 18S and 28S. A small polymorphism was found in ITS1 and ITS2 sequences, but it only partially coincided with the division to pathotypes.

Keywords: clubroot, *Plasmodiophora brassicae*, oilseed rape, Internal Transcribed Spacer

Session 3
Poster 56

Novel lineage of tumorigenic bacteria causing cane gall of blackberry

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Tumorigenic bacteria belonging to the family *Rhizobiaceae* are responsible for crown gall and cane gall disease of various fruit species and may cause significant economic losses in orchards and nurseries. While tumorigenic strains of *Agrobacterium tumefaciens* species complex and *Rhizobium rhizogenes* were predominantly isolated from plants showing crown gall symptoms, *Agrobacterium rubi* is recognized as a causal agent of cane gall disease of *Rubus* spp. The objective of this study was to characterize atypical strains isolated from blackberry showing cane gall symptoms.
Bacterial strains were isolated from tumor tissue of diseased plants collected in two localities in Serbia during 2015-2016. Based on PCR analysis, it was determined that they carry tumor-inducing (Ti) plasmid. Tumorigenicity of strains was confirmed by pathogenicity assay on several test plants. Phylogenetic reconstruction based on 16S rRNA gene placed them within the genus *Rhizobium*, on the same branch as *Rhizobium tubonense*. Multilocus sequence analysis (MLSA) based on the partial sequences of *atpD*, *recA* and *rpoB* housekeeping genes, as well as whole-genome comparisons and calculation of average nucleotide identity (ANI) indicated that these strains represent a new *Rhizobium* species, with *Rhizobium tubonense* as their closest relative.
Overall, our results revealed the existence of additional taxonomic diversity within tumorigenic bacteria. Naturally occurring tumorigenic strains were so far detected only within the genus *Agrobacterium*, and species *Allorhizobium vitis* and *R. rhizogenes*. Additional investigations are ongoing in order to further characterize a novel group of atypical strains.

Keywords: cane gall, blackberry, tumorigenic bacteria, *Rhizobiaceae*, phylogeny, taxonomy

Session 3
Poster 57

Emergence of *Xylella fastidiosa* in Europe: detection on vectors

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Xylella fastidiosa is a xylem limited bacterium originating from the Americas present on a wide range of host plants. *X. fastidiosa* is now an emerging organism in Asia and Europe Since 2013, the subspecies *pauca* is present on olive tree in Italy and since July 2015 two strains of the subspecies *multiplex* have been identified in Corsica on *Polygala myrtifolia,* then on other ornamental and wild plants. In October 2015 the first outbreak was identified in the French Riviera region with the presence of the subspecies *multiplex*. The subspecies *pauca* was detected in Menton in 2016. The subspecies *fastidiosa* was found on Oleander in Germany and the subspecies *fastidiosa, multiplex* and *pauca* are presents on Balearic Islands.

Since 2015, a French official method (MA039 available on www.anses.fr) allows the detection of *X. fastidiosa* on plants by real time PCR after DNA extraction using a commercial kit and an automated system. The determination of the subspecies is carried out by MLST (Yuan *et al*., 2010).

Long-distance dispersal of *X. fastidiosa* depends mainly on the human-mediated movement of infected plants and propagating material but naturally spread on short distances is made by sap-feeding insects. So, detecting the bacteria inside the vector is critical. Adaptation of the protocol used on plants was performed to get an efficient detection test, usable on the known vector *Philaenus spumarius*. This protocol allowed detecting infected *Philaenus spumarius* from Corsican and Apulian outbreaks. Complementary works are in progress to validate the protocol on other potential vectors of *X. fastidiosa.*

Moreover, a test performance study will be organized in 2017 as part of a European Euphresco project involving 12 countries on the detection of *X. fastidiosa* on insects in order to assess different tools (LAMP, real time and regular PCR) and to select the most effective method.

Keywords: *Xylella fastidiosa*, vector, PCR detection, *Philaenus spumarius*

Session 3
Poster 58

Variance in depsipeptide biosynthetic potential among phyto- and entomopathogenic representatives of *Hypocreales*

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Fungi are among the most prolific producers of bioactive secondary metabolites in the biosphere. Among the secondary metabolites, short cyclic or linear peptides are synthesized by modular megasynthases (NRPS, non-ribosomal peptide synthases), originally of bacterial origin. We investigated the multiple producers of different cyclic depsipeptides (enniatins, beauvericins, bassianolide) among entomopathogenic (*Beauveria*, *Metarhizium*, *Lecanicillium, Isaria*), phytopathogenic (*Fusarium*), as well as mycoparasitic (*Trichoderma*) representatives of ecologically diverse *Hypocreales* order.

The results of profiling over 90 potential depsipeptide producing isolates through chemical analyses (LC-MS), as well as degenerate PCR marker amplification and sequencing are shown in conjunction with bioinformatic and phylogenetic analysis. The investigation of extant genetic diversity underlying differences in chemotypes yields novel questions as to the mode of inheritance of depsipeptide synthase modules among beauvericin/enniatin vs. bassianolide producing taxa.

Our analyses have shown structures similarities between enniatin/beauvericin synthase and bassianolide synthase in fungi from *Beauveria* and *Lecanicillium* genera. Although similar modules have been found also in other *Hypocreales*, bassianolide synthesis was only confirmed for *Beauveria* and *Lecanicillium*. The accumulated results provide a better framework for future studies of regulatory divergence between secondary metabolism pathways of higher fungi occupying different ecological niches.

Keywords: cyclodepsipeptides, enniatnis, beauvericins, bassianolide, *Hypocreales*

Research supported by the National Science Centre OPUS 8 grant: NCN 2014/15/B/NZ9/01544

Session 3
Poster 59

Unravelling the mechanisms of *Colletotrichum lupini* host specialization

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Colletotrichum acutatum species complex is considered an important pathogen for several economically important plant species as it causes anthracnose, a disease that can result in death of the host. Since its first diagnose on Lupin in 1939, anthracnose, caused by the see- (and soil-) borne *Colletotrichum lupini*, has become a severe disease of Lupin worldwide, causing meaningful yield losses as high as 100% and becoming a main limiting factor for production. Several morphological, cultural and molecular data confirm *C. lupini* as part of the *C. acutatum* species complex. Although the *C. acutatum* species are considered polyphagous, *C. lupini* has been shown to have a strict host specialization that makes it a fascinating model for evolutionary and biomolecular studies.

With the aim to deeply investigate the infection process of Lupin by *C. lupini*, for the first time an efficient artificial inoculation protocol of Lupin seeds have been set up by using the reference isolate IMI504893. Its genome was sequenced and annotated and the isolate was submitted, for the first time, to *Agrobacterium*-mediated transformation in order to obtain a GFP marked isolate to be used as a tool to better understand the infection process on Lupin hypocotyls.

Five stable transformants have been morphologically and physiologically characterized and a phenotypic microarray test (Biolog) was performed in order to detect metabolic differences between the GFP marked strains and its wt. Information were also exploited to select one transformed strain to be used in host-pathogen interaction studies. Following the artificial inoculation by the GFP marked strain, fluorescence microscopy observations allowed us to describe, step by step, the infection and colonization process. The definition of the timing for host penetration paves the way for further analysis such as a transcriptomic analysis of the *C. lupini*/lupin interaction.

Keywords: *Colletotrichum lupini*, *Lupinus albus*, *anthracnose*, GFP transformant, artificial inoculation

Session 3
Poster 60

Identification of Meloidogyne species on oriental melon in Korea

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Root-knot nematode is wide spread and cause much damage to oriental melon (Cucumis melo var. makuwa) in Korea. When crop rotation is used as a control measure, accurate identification of species of *Meloidogyne* is crucial. This study was conducted to identify *Meloidogyne* species on oriental melon in Korea. PCR-RFLP analysis of 47 Meloidogyne populations was performed using single female. The region between COⅡ and 16S rRNA of the mitochondrial DNA was PCR amplified. Forty six populations produced a single fragment at 1,700bp, and 1 population produced a single fragment at 1,100bp. For the identification more correctly, PCR products were digested with *Hinf*Ⅰ. Thirty four populations were identified as *M. incognita* by generating 1300bp and 400bp fragments. Thirteen populations was not digested with *Hinf*Ⅰ. It is evident that *M. arenania* of a single fragment of 1700bp with no digestion with *Hinf*Ⅰ is wide spread at oriental melon field in Korea which may posesses additional parts in the site of COII/16S rRNA and is genetically different from North American isolates of *M. arenaira*.

Keywords: oriental melon (*Cucumis melo* var. makuwa*),* Root-knot nematodes, species identification, morphology, PCR, RFLP

Session 3
Poster 61

Potential of *Trichoderma spp.* strains isolated from natural sources to produce ceratoplatanins

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Fungi belonging to Trichoderma genus are non-pathogenic soil-borne microorganisms that colonize the root of plants as avirulent plant symbionts. Various species of *Trichoderma* have the ability to inhibit the development of plant pathogenic fungi and even to destroy such organisms through mycoparasitism and other mechanisms. Moreover, they are able to induce the plant's immune system by production of specific elicitors. Among these elicitors, cerato-platanins produced by species of *Trichoderma* are intensively studied in the last years. The aim of this work was to identify new isolates of *Trichoderma spp.* with antifungal properties, to detect the presence of the genes involved in cerato-platanin (CP) biosynthesis and to evaluate their impact on mycoparasitism.
The identification of the *Trichoderma* strains isolated from compost was realized with specie-specific primers and allowed the identification of *T.atroviride, T,asperellum, T.harzianum* and *T.longibrachiatum* species. Intraspecific genomic variability was examined by RAPD and with ISSR primers. Differences in electrophoretic patterns among the strains belonging to the same species were detected. The detection of *epl1* gene involved in biosynthesis of CP was performed with primers proposed in literature. Direct confrontation assays and microscopically analysis were used to evaluate the antifungal action of selected *Trichoderma* strains against *Rhizoctonia solani, Fusarium oxysporum, Sclerotium bataticola, Botrytis cinerea* and the influence of CP coding genes on mycoparasitism. Amplicons with desired length, corresponding to *epl1* gene, were observed in strains of *T.harzianum, T.atroviride* and *T.asperellum*. Differences among strains with or without *epl1* gene regarding the inhibition of plant pathogens were revealed suggesting the possible involvement of EPL1 protein in the interactions between the antagonist and the target.
These results contribute to the understanding of the complex mechanisms of the interactions between biocontrol *Trichoderma* species and plant pathogenic fungi and sustain the use of compost as a sources of biocontrol microorganisms for plant protection.

Keywords: cerato-platanins, *Trichoderma*, molecular detection

Session 3
Poster 62

First report of *Acidovorax citrulli* on melon in Guadeloupe

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Acidovorax citrulli (Schaad *et al*., 1978; Schaad *et al*., 2009), a gram-negative bacterium, is the causal agent of bacterial fruit blotch (BFB) of cucurbit plants. Its main hosts are watermelon and melon, but cucumber, pumpkin and squash are also described as host plants. As it is a seed born disease, the seeds are likely to be responsible for spreading over long distances, mainly under warm climate. This bacterium is not a quarantine organism for EU but it was added to the EPPO A1 list since 2014 due to the risk of emergence around the Mediterranean Basin. In America, it has been present since the 1990s (first in Florida in 1989). Its impact on the production of marketable fruits can be catastrophic with losses of more than 90%. Then the disease has spread to many parts of the world (Asia). Absent from Europe until 2005 (first description in Greece, Holeva *et al*., 2009), it was declared present with a restricted distribution or sporadic outbreaks in Greece, Hungary (Palkovics L *et al*., 2008), Turkey (Mirik *et al.,* 2006), Israel (Burdman *et al*., 2005), Italy, Serbia (Popovic and Ivanovic, 2015); it is now considered eradicated in these countries.
In May 2015, *A. citrulli* was detected in Guadeloupe, in French West Indies, on symptomatic melon. The melon presented water-soaked lesions on leaves and fruits and bacterium was isolated from both. Identification of the isolates was performed by biochemical and molecular tests (Bahar *et al*., 2008). The pathogenicity of the isolates was verified and Koch's postulate demonstrated.
This first report shows the risk of emergence of new pathogens under hot and humid climate and the importance of using healthy seeds.

Keywords: *Acidovorax citrulli*, symptomatic plants, cucurbit seeds, isolation, PCR, emergence

Session 3
Poster 63

New foliar and soil-borne pathogens recently observed on leafy vegetables in Europe

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During the past years new diseases caused by soil-borne and foliar pathogens were observed for the first time in Europe on lettuce, rocket, spinach and lamb's lettuce grown mainly for the ready to eat sector. Italy is the second producer in Europe of fresh-cut leafy vegetables, representing indeed a very interesting case study from a phytopathological point of view. Among foliar diseases, *Fusarium equiseti* on wild and cultivated rocket and lettuce, *Allophoma tropica* on lettuce, *Colletotrichum kahawae* on cultivated rocket, *Myrothecium roridum* on lamb's lettuce and *M. verrucaria* on spinach and wild rocket were observed. Moreover, a new race of *Fusarium oxysporum* f.sp. *lactucae* has been isolated for the first time in the Netherlands on affected lettuce plants, while, race 1 of this pathogen has been found recently in France. Some of these new pathogens have been isolated from seeds. The symptoms of the diseases caused by these fungal pathogens, the biology and physiological characteristics of the causal agents and some information concerning their epidemiology are presented.

Keywords: leafy vegetables, alien pathogens, seed-borne pathogens, Emphasis project

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Seed transmission of Tomato yellow leaf curl virus in sweet peppers (*Capsicum annuum*)

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The sweet pepper (*Capsicum annuum*) is a popular crop and an asymptomatic host of Tomato yellow leaf curl virus (TYLCV). A previous study showed that TYLCV could be transmitted by the seeds of tomato plants, but this phenomenon has not been confirmed in other plants. In 2015, four different cultivars of sweet pepper ('Super Yellow,' 'Super Red,' 'Sunnyez' and 'Cupra') confirmed to be susceptible to TYLCV by the previous study were planted and inoculated with agrobacterium containing a TYLCV infectious clone at an isolated greenhouse. Three months after inoculation, the leaves of the 'Super Yellow' cultivar showed 80% (8/10) infectivity and the other three sweet pepper cultivars showed 30 to 50% infectivity. All of the 'Super Yellow' seed bunches (five seeds per bunch) from plants whose leaves were confirmed to be TYLCV-infected were also TYLCV-infected (8/8). The seeds of other cultivars showed 20 to 40% infectivity. Virus dissemination rates were also verified with 10 bunches of seedlings for each cultivar (five seedlings per pool). Eight bunches of 'Super Yellow' seedlings (8/10) were confirmed to be TYLCV-infected and one to three bunches of each of the other cultivar seedlings were also infected. Viral replication in TYLCV-infected seeds and seedlings was confirmed via strand-specific amplification using virion-sense- and complementary-sense-specific primer sets. This is the first report of TYLCV seed transmission in sweet pepper plants and among non-tomato plants. Because the sweet pepper plant is an asymptomatic host of TYLCV, sweet pepper seeds infected with TYLCV could act as a silent invader of tomatoes and other host crops; therefore, it is particularly important to identify TYLCV seed transmission in non-tomato hosts.

Keywords: Begomovirus, geminivirus, seed transmission, sweet pepper, Tomato yellow leaf curl virus

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First report of seed transmission of *Tomato yellow leaf curl virus* in white soybean (*Glycine max*)

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Tomato yellow leaf curl virus (TYLCV) causes damage to several economically important crops such as tomatoes, peppers and cucurbits. TYLCV infection of common bean (*Phaseolus vulgaris*) was reported previously, but soybean (*Glycine max*) has not previously been identified as a TYLCV host. In 2015, five cultivars of white soybean ('Jinpung,' 'Taegwang,' 'Seonpung,' 'Neulchan' and 'Daepung') were agro-inoculated using an infectious TYLCV clone in an isolated plant cultivation chamber in Wanju. Thirty days post-inoculation, 'Jinpung' and 'Taegwang' showed 86% and 100% infectivity, respectively, and other soybean cultivars showed 25 to 66% infectivity. Typical TYLCV symptoms were not observed in any inoculated plants. Viral replication in TYLCV-infected leaves was confirmed by the existence of double-stranded viral DNA with strand-specific amplification using the virion-sense- and complementary-sense-specific primer sets introduced in the previous study. Based on a previous study about seed transmission of TYLCV in tomato plants, seeds from TYLCV-inoculated soybean plants confirmed to be TYLCV-infected were harvested and DNA was isolated from bundles of five randomly selected seeds and amplified with a TYLCV-specific primer set in order to verify TYLCV seed transmission in soybean. Except for one bunch of 'Jinpung' seeds, all bundles of seeds from infected soybean plants were verified to be TYLCV-infected (13 of 14 bunches). Virus dissemination rates were also confirmed from three bunches of seedlings out of 14 bunches germinated from infected seeds (five seedlings per bunch). Viral replication was also identified in seeds and seedlings confirmed to be TYLCV-infected via strand-specific amplification as previously described (Edgar et al. 2014). This is the first report proving that soybean (*G. max*) is a TYLCV host and that TYLCV is a seed-transmissible virus in white soybean.

Keywords: Begomovirus, geminivirus, reservoir, seed transmission, soybean, *Tomato yellow leaf curl virus*

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Poster 66

First report of *Tomato yellow leaf curl virus* infecting monocotyledonous plants

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Tomato yellow leaf curl virus (TYLCV) is one of the most important plant viruses belonging to the genus *Begomovirus* of the family *Geminiviridae*. From 2013 to 2014, to identify natural weed hosts that could act as reservoirs of TYLCV, one hundred samples were collected at a TYLCV-infected tomato-cultivating farm in Iksan and identified as belonging to 40 species from 18 families. Among them, TYLCV was detected from 57 smaples belonging to 28 species by PCR using root samples including five species (*Eleusine indica*, *Digitaria ciliaris*, *Echinochloa crus-galli*, *Panicum dichotomiflorum* and *Setaria faberi*) from the family Poaceae. Whitefly (*Bemisia tabaci*)-mediated TYLCV transmission from TYLCV-infected *E. indica* plant to healthy tomatoes was confirmed, and inoculated tomatoes showed typical symptoms such as leaf curling and yellowing. In addition, TYLCV was also detected in leaf and root samples of *E. indica* plants inoculated by both whitefly-mediated transmission using TYLCV-viruliferous whitefly and agro-inoculation using the TYLCV infectious clone. The majority of mastreviruses infect monocotyledonous plants, but few dicotyledonous plants infected with masteviruses have been reported. However, no exception was reported among begomoviruses known as infecting dicots only. This is the first report of begomovirus-infecting monocotyledonous plants.

Keywords: Begomovirus, *Eleusine indica*, geminivirus, monocotyledonous plants, *Tomato yellow leaf curl virus*

Session 3
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Emerging sea buckthorn diseases in Latvia and associated fungal pathogens

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Sea buckthorn (*Hippophae rhamnoides* L.) is an important fruit crop in Latvia and its area under cultivation is rapidly expanding. In general, sea buckthorn diseases and their causal agents have been poorly studied, limited to only a few records of stem canker, wilt and dry shrink diseases caused by *Stigmina* sp, *Verticillium* spp., and *Fusarium* spp., respectively. In Latvia, concerns of growers have raised on diseases spreading in sea buckthorn plantations. To identify and characterize sea buckthorn diseases prevailing in Latvia 55 locations including commercial orchards and wild habitats were surveyed. The samples from branches, roots, and trunks with various disease symptoms were collected. Fungi were isolated from surface sterilized plant tissues on potato dextrose agar, sub-cultured in pure cultures and preserved for further studies. The isolated fungi were characterized and identified by morphological characters, sequencing of the ITS region and comparison to other related fungi by phylogenetic analysis. During the surveys, overall decline, wilt, severe canker and dieback symptoms often causing a death of the plants was observed. The fungal isolates obtained from infected tissues were identified as belonging to the genera of *Diaporthe*, *Eutypa*, *Fusarium*, *Valsa* and *Verticillium* which are the well-known causal agents of wilts, cankers, and diebacks on various trees. Besides, a range of other fungi was obtained which appeared to be less-known and therefore their roles in sea buckthorn diseases have to be further investigated. The pathogenicity evaluation of the fungal isolates on sea buckthorn plants in a greenhouse is currently under development.

Keywords: *Hippophae rhamnoides*, fungal diseases, canker, wilt

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Poster 68

Fusarium species associated with roots of leguminous plants grown in different environmental regions of Europe

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Growing legumes as cover crops, intercropped or under-sown with cereals can provide multiple beneficial services to agro-ecosystems. However, agronomic benefits of their inclusion into the rotations can only become effective if the pathological risks for crop rotations are known and rotations designed accordingly. This study brings together findings from surveys conducted in 2013 and 2014 across five European sites in Switzerland, Italy, Germany, and Sweden. Incidence and characterization of *Fusarium* spp. associated with the roots of two clover and two vetch species grown as cover crops or under-sown in wheat were determined.
Out of 1480 roots analyzed in both years, 670 *Fusarium* isolates were obtained. No strong separation among the sites could be observed in *Fusarium* community structure suggesting that each site was dominated by similar species. The species richness curve for both clover species was much steeper compared to vetch species showing some host effects on the *Fusarium* community composition. The most frequently isolated species in both years from all sites and all four hosts were *F. oxysporum* and *F. avenaceum*. In pathogenicity tests on peas isolates of *F. avenaceum* caused highest biomass reductions and most severe root rot symptoms, followed by isolates *of F. oxysporum, F. solani* and non to weakly pathogenic isolates of *F. tricinctum, F. acuminatum* and *F. equiseti*. The prevalence of *F. avenaceum* and the potential of isolates to cause severe yield reductions under favorable conditions suggest that it could emerge as a potential risk under legume rich crop rotations.

Keywords: clover, vetch, *Fusarium*, legume root pathogens

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Interactions of pea pathogens with potentially useful leguminous cover crop species

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Modern cropping systems mainly rely on growing a narrow range of crop species and genotypes while the possibility of using novel crops, particularly leguminous species, which have the potential to play a major role in more diversified and sustainable food production systems, has been neglected. These species could provide multiple beneficial services to agroecosystems when used as cover crops (CC), such as green manures or living mulch. Before introduction, it is important, however, to assess if such species share important difficult to manage pathogens of main crops.
A total of 62 accessions belonging to ten legume genera were screened under controlled conditions for their susceptibility/resistance to selected pea pathogenic isolates of *Fusarium avenaceum, F. oxysporum, F. solani, Peyronellaea pinodella (syn. Phoma medicaginis)* and *Didymella pinodes,* all major pea root pathogens. The plants were inoculated with $2x10^4$ spores g^{-1} substrate one day after transplanting pre-germinated seeds. Three pea varieties, the resistant *EFB 33* and the susceptible *Santana* and a Brazilan accession, were included as additional controls. Five weeks after sowing, disease symptoms were assessed and plant growth parameters measured. Almost all plant species and accessions tested were highly susceptible to *Fusarium avenaceum*, with notable exceptions of *Crotalaria ochroleuca, Lotus pedunculatus* and a few *Trifolium* and *Medicago* accessions. *F. oxysporum* caused variable disease severity on some *Trifolium* species, otherwise, infections were low, while *F. solani* caused overall higher disease severity with some variation among accessions. This suggests specific interactions. *Peyronellaea pinodella* and *Didymella pinodes* most severely affected *Lathyrus*, otherwise infections were low with *D. pinodes* a pathogen that apparently is relatively host specific. Integration of this knowledge is crucially important in the design of rotations with cover crops in order to integrate them successfully and sustainably into agricultural systems as required by the EU greening policy.

Keywords: Pea root rot, cover crops, EU greening, crop rotation

Session 3
Poster 70

Report and characterization of bacterial diseases caused by *Xanthomonas oryzae* in Senegal

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Over the last decades rice cultivation has been increasing drastically in Africa. Rice (*Oryza sativa*) is becoming the number one crop produced for human consumption feeding more than half of the population in Africa. Among factors that limit or reduce rice agricultural yield in Africa, are bacterial diseases that impact food security. *Xanthomonas oryzae* cause important yield losses of rice. Both bacterial blight (BB) and bacterial leaf steak (BLS) are reported in various countries in Africa. *Xanthomonas oryzae* pv. *oryzae* (*Xoo,* causal agent of BB) was reported in Senegal in the eighties by Trinh et al. Trinh did not isolate strains of *Xoo* and since no other report has been made. *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*, causal agent of BLS) was not reported in Senegal so far. The use of rice cultivars with introgressed disease resistance (R) genes is currently the best way to control BB disease with minimal environmental effects and cost. No rice resistance gene has been reported to control BLS worldwide. Resistance breeding depends on harnessing genetic diversity of pathogen populations. The choice of BB and BLS resistance gene(s) should be made based upon their effectiveness against the prevalent races of *Xoo* and most virulent strains of *Xoc* in the region. As no strains of *Xoo* and *Xoc* were collected and characterized in Senegal so far, no strategy has been pursued to control the diseases. Here, we aim at confirming Trinh's observations (BB presence). To that purpose we surveyed the presence and prevalence of BB in different regions of Senegal. At the same time we look at the presence of BLS. Fields in main areas of rice production in Senegal were monitored between 2014 and 2016. Leaf samples were collected and analyzed. After bacterial isolation, a PCR multiplex was used to confirm the presence of *Xanthomonas oryzae* pv. oryzae and *oryzicola* in different sites of each region. A set of IRBB lines each carrying a single R gene was used to identifying the race of *Xoo* in presence in Senegal. We also assessed the virulence of *Xoc* strains on a susceptible rice cultivar. We will present and discuss the results obtained during this two year's survey in Senegal.

Session 3
Poster 71

Characterization and pathogenicity of *Biscogniauxia mediterranea* **associated with cork oak (***Quercus suber L.* **) in Tunisia.**

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Biscogniauxia mediterranea is a virulent fungal pathogen involved in oak decline and caused high mortality in cork oak trees in the mediterranean region. It is responsable of charcoal canker disease. The occurrence of *B.mediterranea* associated with cork oak in Tunisia has been observed since the sixties. Therefore, the present study was aimed to characterize *B.mediterannea* isolated from different organs of cork oak trees in a natural forest ecosystem in Tunisia and to evaluate its pathogenicity.
B.mediterranea isolates were obtained from leaves, brunches and trunks of ten declining trees. Morphological characterization was based on the mycelial growth rate and the colony pigmentation. Molecular identification was performed by PCR amplification of the fungal DNA with the specific primers MED1/MED2. Pathogenicity test was conducted on young cork oak plantations of 2 years old maintained in a greenhouse for 2 months.
A total of 70 isolates of *B. mediterranea* was obtained. All the organs were revealed to be infected by *B.mediterranea*. However, the statistical analysis showed a significant variation of the isolation frequency between the different organs. The Brunches were shown to be the most infected. For the obtained isolates, PCR amplification with the primers MED1/MED2 generated only one PCR product of approximately 380 pb. Thus, all isolates were confirmed as *B.mediterranea* species. Pathogenicity test confirmed the virulence of the tested *B.mediterranea* isolates. These results emphasize the necessity for *further in-depth studies on the fungus biology and the epidemiology of charcoal canker in tunisian forests.*

Keywords: *Biscogniauxia mediterranea*, characterization, pathogenicity, cork oak

Session 3
Poster 72

A new *Tobamovirus* isolate infects tomato plants harboring Tm-22 resistance genes - a potential global threat to tomato production

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An outbreak of a new disease infecting tomatoes ocurred in October-November 2014 in Southern Israel. Symptomatic plants showed a mosaic pattern on leaves accompanied occasionally by narrowing of leaves and yellow spotted fruit. The disease spread mechanically and rapidly reminiscent of tobamovirus infection. Epidemiological studies showed the spread of the disease in various growing areas, in the South and towards the Southeast and Northern parts of the country within a year. Transmission electron microscope (TEM) analysis showed a single rod-like form characteristic to the Tobamovirus genus. We confirmed Koch's postulates for the disease followed by partial host range determination and revealed that tomato cultivars certified to harbor the Tm-22 resistance gene, are susceptible to the new viral disease. We further characterized the viral source of the disease using a range of antisera for serological detection and analyzed various virus genera and families for cross-reactivity with the virus. Next generation sequencing of total small RNA was performed on two cultivars grown in two different locations. The complete genome sequence of the new Israeli tobamovirus showed high sequence identity to the Jordanian isolate of tomato brown rugose fruit virus.

Keywords: Tm-22, next-generation sequencing (NGS)

SESSION 4. MICROBIAL DETERMINANTS OF PATHOGEN AND SYMBIOTIC INTERACTIONS

Session 4
Poster 73

Verticillium wilt on fiber flax: symptoms and pathogen development *in planta*

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Fiber flax (*Linum usitatissimum* L.), an important crop in Normandy (France), is increasingly affected by Verticillium wilt caused by the soil-borne fungus *Verticillium dahliae* Kleb. This disease leads to non-negligible yield losses and depreciated fibers that are consequently difficult to upgrade. Verticillium wilt is a major threat to a large broad range of agriculture; in this study, fiber flax susceptible cv. Adélie was infected by Vd05-833 (isolated on fiber flax, this study) or green fluorescent protein-tagged VdLs17 (isolated on lettuce, ATCC accession MYA-4575). Between three and four weeks post inoculation, wilting symptoms on leaves were first observed, with acropetal growth during the following weeks. Pathogen development was tracked by confocal microscopy during the asymptomatic and symptomatic stages. First, conidia germination led to the development of hyphae on root epidermis more particularly on zone of cell differentiation and around emerging lateral roots while the zone of cell division and the root tip were free of the pathogen. At three days post inoculation, the zone of cell differentiation and lateral roots were embedded into a fungal mass. Swelling structures such as appressoria, were observed at one week post inoculation. At two weeks post inoculation, the pathogen had colonized xylem vessels in roots, followed by the stem and finally leaves during the symptomatic stage. Pathogen quantity was assessed by real-time PCR during the first 30 dpi. A significant decrease in fungal DNA was detected between the first days when the pathogen was spread onto the root (asymptomatic phase) and 30 dpi, when symptoms appeared and the pathogen colonized vascular tissues. All of these results provide a global account of the *V. dahliae* disease cycle when infecting fiber flax.

Keywords: fiber flax, Verticillium wilt, GFP confocal microscopy, real-time PCR.

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Poster 74

Comparative transcriptomic study of two grapevine fanleaf virus strains inducing contrasted symptoms on *Vitis vinifera* cv. Gewurztraminer

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Grapevine fanleaf virus (GFLV) is the major virus inducing fanleaf degeneration, one of the most harmful viral diseases of *Vitis spp* worldwide. In the French vineyard, the losses due to this endemic disease are estimated between 350 and 850 million euros per year. Symptoms depend on environment conditions, rootstock-scion combinations and virus strains. They include leaf and cane malformations, yellow mosaic, small fruit clusters, and a progressive decline that eventually leads to death. Little is known about the determinism and the mechanisms of symptom induction by GFLV on its natural host.

To gain knowledge on virus determinants and pathogenesis mechanisms we established a trial with Gewurztraminer plants infected with single GFLV strain F13 or B844. GFLV inoculation was performed by *in vitro* heterologous grafting. The trial took place in an experimental isolated vineyard away from superinfection. These plants were monitored from 2012 to 2014 for symptoms, fruit yield and quality (Vigne *et al.* 2015). GFLV-F13 infected grapevines showed discrete foliar symptoms, whereas GFLV-B844 infected plants displayed a severe degeneration of the vine stock.

We took advantage of these contrasting symptoms in a non-targeted approach. To this end, leaves were sampled during the 2016 vegetative season and total RNAs were extracted from three B844- or F13-infected Gewurztraminer. A transcriptomic analysis by RNAseq was initiated to identify deregulated genes and deduce the major pathways involved in symptom formation. The first results will be presented and discussed.

Keywords: symptomatology, Nepovirus, RNAseq, Vitis

Session 4
Poster 75

Analysis of *Venturia inaequalis* genes expression profiles: searching for the genes associated with apple-scab-resistance breakdown

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Apple scab caused by *Venturia inaequalis* (Cooke) Wint is one of the most serious diseases of apple (*Malus* x *domestica*). The naturally existing scab resistance in some apple cultivars has been recognized as the preferred sustainable alternative to chemical control. Two main factors involved in gene-for-gene relation are: genes or gene 'R' at the host side and compatible *avr* locus present in pathogen, responsible for avirulence toward the apple cultivars. Seventeen different *V. inaequalis* races have been identified so far, each overcoming one major monogenic resistance source in *Malus* spp. cultivars. It is expected that each different host-specificity of the race results not only from different gene content but also from different gene expression profile and involvement of different effector proteins. The aim of the study was to identify candidate genes associated with apple-scab-resistance breakdown via transcriptome analysis with RNA-seq technique of two races of *V. inaequalis* in two susceptible cultivars.
Gene expression profile of *V. inaequalis* strains, representing races able to infect 'Topaz' and 'Golden Delicious' cultivars of apple (containing *Rvi6* and *Rvi1* genes, respectively) has been analysed *in planta* and *in vitro* during this study.
Sporulating scab lesions on apple leaves and mycelium respectively growing on PDA of both races were excised and total RNA was extracted from them. cDNA libraries have been constructed from polyadenylated RNA fraction and sequenced on Illumina HiSeq™ NGS platform. Obtained reads were mapped to *Malus* and *V. inaequalis* reference genomes. The subset of reads, which did not mapped to *Malus* reference, has been assembled *de novo.* More than 24 000 contigs obtained as a result of the assembly have been used for calculation of expression profile for each race separately. Each contig has been sorted to one of the 15 sets representing all possible expression patterns. Currently, about 1500 contigs potentially involved in pathogenicity process have been preliminary selected for further analysis. Among them 122 contigs showed especially promising patterns, which will be verified using RT-rtPCR. Our long term goal is to identify *V. inaequalis* genes responsible for overcoming plant defence based on *Rvi6* and *Rvi1* apple scab resistance genes of *Malus* host.
This work was financed by National Science Centre, Poland grant No. UMO-2013/09/B/NZ9/02343.

Keywords: gene expression profiling, gene for gene interaction, RNA sequencing

Session 4
Poster 76

Genome-wide analysis of *Corynespora cassiicola* putative effectors involved in the CLF disease of rubber tree

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Corynespora cassiicola is an Ascomycete with a large host range, mostly in the plant kingdom. It was also reported in other fungi, nematodes and in rare cases of human disease. It was mostly described as a necrotroph, but also as an endophyte or a saprobe. The species was previously placed in the Fungal Tree of Life where it formed a distinct clade among the Pleosporales, together with *Corynespora smithii*. Several studies have demonstrated the important genetic diversity among the species.

In rubber tree, it is responsible for the Corynespora Leaf fall (CLF) disease which causes massive defoliations on susceptible cultivars, thus impairing rubber production. We have previously purified and characterized a small protein toxin, secreted by the highly virulent rubber tree isolate CCP and playing a role in virulence. However the existence of other effectors, yet uncharacterized, was evidenced.

The objective of the present study was to identify *in silico* all potential effectors involved in CLF. The genome of our reference isolate CCP was sequenced and assembled by DOE-Joint Genome Institute in frame of the 1000 Fungal Genome project, and putative effectors identified. PCA based on the composition in effectors of 45 fungal species was only weakly related to phylogeny. However, *C. cassiicola* was found associated with species sharing common life style features, ie large host range and diverse trophic capacities. Transcripts profiling was conducted to identify functional effectors differentially expressed during the compatible interaction with rubber tree. Finally, intraspecific comparative analysis of 37 newly-assembled *C. cassiicola* genomes was conducted in order to compare their respective sets of putative effectors. These results will be discussed in relation with the genetic diversity and the known physiological specificities of the studied isolates.

Keywords: *Corynespora cassiicola, Hevea brasilensis,* genomics, transcriptomics, effectors, Cassiicolin

Session 4
Poster 77

Comparative genomics identifies a new translocon candidate in the type III secretion system of Gram-negative bacteria

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Xanthomonas translucens is the causal agent of bacterial leaf streak, the most common bacterial disease of wheat and barley. To cause disease, most xanthomonads depend on a highly conserved type III secretion system, which translocates type III effectors into host plant cells. Mutagenesis of the conserved type III secretion gene *hrcT* confirmed that the *X. translucens* type III secretion system is required to cause disease on the host plant barley and to trigger a non-host hypersensitive response in pepper leaves. Type III effectors are delivered to the host cell by a surface appendage, the hollow Hrp pilus, and a translocon protein complex that inserts into the plant cell plasma membrane. Homologs of the *Xanthomonas* HrpF protein, including PopF from *Ralstonia solanacearum* and NolX from rhizobia, are thought to act as a translocon protein. Comparative genomics revealed that *X. translucens* strains harbor a noncanonical *hrp* gene cluster, which rather shares features with type III secretion systems from *Ralstonia solanacearum*, *Paraburkholderia andropogonis*, *Collimonas fungivorans* and *Uliginosibacterium gangwonense* than other *Xanthomonas* spp. Surprisingly, none of these bacteria, except *R. solanacearum*, encode a homolog of the HrpF translocon. Here, we aimed at identifying a candidate translocon from *X. translucens*. Notably, genomes from strains that lacked *hrpF*/*popF*/*nolX* instead encode another gene, called *hgiA* (for *hrpG*-induced), adjacent to and likely co-regulated with the type III secretion system gene cluster. An insertional mutant in the *X. translucens hgiA* gene, which is the first gene of a two-gene operon, *hgiA*-*hpaH*, was non-pathogenic on barley and did not cause the hypersensitive response or programmed cell death in non-host pepper similar to the *hrcT* mutant. The *hgiA* mutant phenotypes were partially complemented by either *hgiA* or the downstream gene, *hpaH*, which has been described as a facilitator of translocation in *Xanthomonas oryzae*. These findings suggest that both HgiA and HpaH may contribute to the injection of type III effectors into plant cells.

Keywords: *Xanthomonas translucens*, type III secretion system, type III effector, translocon, HrpF protein, barley

Session 4
Poster 78

A deepen knowledge of *Colletotrichum lupini*, a major threat for Lupin cultures

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Colletotrichum is a fungal genus gathering numerous species causing anthracnose on a large host range and these pathogens are spread worldwide. Within the *Colletotrichum acutatum* complex, the filamentous fungus *Colletotrichum lupini* is specific to Lupins (*Lupinus* spp.) and responsible for the anthracnose disease, one of the major threats for Lupin crops.
In order to get an in-depth understanding of this fungus, we built an extensive collection of 115 strains including 70 strains isolated from infected Lupins in France and 15 from different countries worldwide distributed, we also included 30 strains from closely related species as outgroups. An extensive genetic characterization based on 10 loci showing high resolution has been carried out in order to investigate the population structure. Based on the results gained from the multilocus analysis, we sequenced and annotated the genomes of two *C. lupini* reference genomes and resequenced three isolates along with 12 closely related *acutatum* species characterized by a polyphagous lifestyle. The genome sequences were used to perform a comparative analysis with the aim of exploring genomic signatures associated with host preference in *C. lupini*.
We provide a first look at the host adaptations at the genomic level that are associated with host specialization in *Colletotrichum* spp. This study also demonstrates the flexibility of *Colletotrichum* genomes, and shows that recent changes in the genomes are associated with major changes in host range and epidemiology. This study also exhibits the need for higher resolution taxonomic sampling in order to better understand the role of gene duplication and loss in the evolution of fungal genomes.
To gain a better insight in the molecular processes involved in the *C. lupini*/Lupin interaction and, to confirm the computational results, we will perform a comparative 'omic approach (transcriptomic and proteomic) with the polyphagous closely related pathogen *C. fioriniae*.

Keywords: *Colletotrichum*, Lupins, genomic analysis, host adaptation

Session 4
Poster 79

Cinnamyl alcohol dehydrogenase deficiency impairs poplar-root-knot nematode interaction

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A well-characterized branch of the phenylpropanoid pathway leads to the biosynthesis of the 3 main monolignols, which are the *p*-coumaryl, coniferyl and sinapyl alcohols. Altered expression of practically all the genes of the monolignol biosynthesis pathway has been analysed in transgenic plants and mutants, but essentially regarding their impact on lignification. Several studies, using transcriptomic, proteomic and/or metabolomics approaches have shown that the alteration of the expression of genes involved in this pathway affects not only the monolignol biosynthesis pathway but also the expression of genes and the accumulation of metabolites belonging to other phenylpropanoid branches. Monolignols can be polymerized into lignins at the cell wall level or converted in lignans, that are di- or oligolignols known for their role in the defense or during plant microorganisms interactions. RKN belonging to the genus Meloidogyne are endoparasites causing considerable damages to both annual and perennial plants worldwide. Global analyses and particularly transcriptomic analyses, in several interactions models have highlighted the phenylpropanoid pathway and particularly monolignol pathway as to be altered during both early and late gall developmental stages. Perennial woody species are a source of a multitude of fruits but also of a huge biomass for the production of wood, pulp and paper or biofuels. One of the current scientific challenges in the context of climate change is on the one hand to better understand how pathogens affect trees and influence their evolution, and on the other hand to identify the molecular basis of the natural resistance of trees towards these pathogens. In this context, we studied the impact of the deficiency of several monolignol biosynthesis steps on the poplar-root-knot nematode interaction. We show that cinnamyl alcohol dehydrogenase (CAD) deficiency impairs poplar-root-knot nematode interaction. Thanks to a multidisciplinary approach regrouping metabolomic, transcriptomic and Raman spectromicroscopy we tried to explain the molecular origin of the impact of the CAD deficiency for plant-root-knot nematode interaction.

Keywords: Root-knot nematode, poplar, cinnamyl alcohol dehydrogenase, metabolism

Session 4
Poster 80

Study of an effector family of *Plasmopara viticola*, the grapevine downy mildew pathogen

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Plasmopara viticola is an obligate biotrophic oomycete responsible for grapevine downy mildew, a very recent disease on the European continent, arriving from America in 1878. This disease causes heavy damage on traditional grapevines and it is currently controlled chemically. To obtain an optimal health status, growers resort to many fungicide treatments. The excessive application of phytosanitary measures on vineyards imposes a selection pressure responsible for the appearance of fungicide-resistant strains.

An alternative solution to expensive and environmentally unfriendly pesticides is the use of resistant grapevine varieties. Traditional European grapevine varieties are susceptible to *P. viticola*, the resistance needs thus to be introduced through breeding programs, from American and Asian resistant Vitaceae.

In plants, most resistance genes (R) recognize a specific product coded by a gene of the pathogen called the avirulence (*Avr*) gene and activate defence responses. Almost all known oomycete Avr proteins belong to the RXLR-EER effector family. These proteins are secreted during plant infection and, thanks to the RXLR amino-acid motif, internalized into plant cells where they manipulate plant defence and promote virulence.

To identify new resistance genes, our strategy is to search for RXLR effectors essential to the virulence of the pathogen and use them to screen resistant grapevines. Using genomic resources, more than 250 *P. viticola* effector candidates have been identified. Among the identified effector candidates, more than sixty are structurally similar to RXLR effectors from other phytopathogenic oomycetes but, surprisingly, they are devoid of RXLR motif. Two of these effectors held our attention, because they are expressed during the infection and their transient expression in leaves of *Nicotiana benthamiana* leads to necrosis. Their study could lead to a better understanding of the evolution of the pathogen's virulence and of the mechanisms of effector's internalization into the plant cells.

Keywords: RXLR effectors, downy mildew, grapevine, *Plasmopara viticola*, oomycete

Session 4
Poster 81

Functional analysis of root-knot nematode (*Meloidogyne javanica*) virulence genes in rice

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Root-knot nematodes (RKN) are endo-parasites with a wide host range, encompassing mono- and dicotyledonous plant crops. Several RKN species are responsible for rice (*Oryza sativa*) production losses in Brazil, Asia or Africa. Successful infection is likely achieved by effector proteins produced in the nematode esophageal gland cells and released in the host plant cells. The aim of this study was to assess the functional role of three *Meloidogyne* secreted proteins (MSP) in rice - nematode interactions. We show that the *Meloidogyne* sp. esophageal gland cell proteins MSP7, MSP18 and MSP19 are conserved in *M. incognita*, *M. javanica* and *M. graminicola* species infecting rice. RT-qPCR assays showed that MSP2, MSP18 and MSP19 genes are over-expressed all along the infection cycle in rice roots. Subcellular localization experiments in onion cells showed that that MSP2 may be addressed to the nucleus, and MSP18 and MSP19 to the cytoplasm and nucleus. Transgenic rice (*O. sativa* Nipponbare) plants expressing the candidate proteins or artificial micro-RNAs (amiRNAs) able to silence the cognate genes in the nematode were produced. Assessment of nematode reproduction on homozygous transgenic lines allowed the identification of rice lineages with altered susceptibility, indicating that these proteins may be involved in *Meloidogyne* virulence. Overexpression of MSP18 in rice enhanced *M. javanica* and *M. graminicola* reproduction, indicating that the MSP18 protein facilitates RKN parasitism. The role of these proteins in suppressing host immunity was investigated in tobacco cell death-induced transient assays. MSP18 suppressed the INF1-triggered programmed cell death, suggesting that MSP18 can interfere with the plant defense pathways. Data obtained may help deciphering nematode-rice molecular interactions and highlight MSP18 as a novel RKN virulence effector able to modulate host immunity.

Keywords: *Meloidogyne*, amiRNA, functional analysis, immuno-modulator, rice, virulence effector

Session 4
Poster 82

Unrelated virulence factors encoded by nematode and viral genomes converge onto HUB proteins in Arabidopsis

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Plants permanently face changing unfavorable environments against which they deploy adapted responses to maintain their life cycle. In turn, plant-invading pathogens need to take into consideration evolving defense responses from their host to counteract them efficiently. Recent studies in Arabidopsis demonstrated that pathogens from different kingdoms such phytopathogenic bacteria and eukaryote obligate oomycetes and ascomycetes, deploy independently evolved virulence proteins that physically interact with a limited set of highly connected protein nodes (hub proteins). Hubs and their interactors constitute protein-protein interaction (PPI) networks with given architecture and biological significance. Therefore, interfering with plant host PPI networks through the targeting of hub proteins is thought to provide to pathogens an efficient mean to manipulate key host functions.
Here, we used a yeast-two hybrid approach on selected plant hub proteins and pathogen effectors to identify two new evolutionary unrelated pathogen families, namely the *rice yellow mottle and Imperata yellow mottle* sobemoviruses, and the root-knot nematode (RKN) *Meloidogyne Incognita*, that are able to converge onto plant hub proteins. We notably identify one hub most probably targeted to promote RKN feeding site at the plant root, and other hub proteins that might govern plant susceptibility to viruses at the leaf surface.

Keywords: plant immunity, Hub proteins, *Arabidopsis*, protein-protein interaction, sobemovirus, root-knot nematode

Session 4
Poster 83

Both GacS-regulated lipopeptides and the type III secretion system contribute to *Pseudomonas cichorii* caused necrosis in hosts

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Pseudomonas cichorii SF1---54, the causal agent of lettuce midrib rot disease, produces several lipopeptides, including cichofactins and cichopeptins which are important virulence factors. Cichofactins are linear lipopeptides and involved in antimicrobial activity and surface motility of *P. cichorii* SF1---54. Cichopeptins are phytotoxic cyclic lipopeptides and able to cause necrotic symptoms on chicory. The GacS/GacA two---component system is well known to regulate production of secondary metabolites including lipopeptides in pseudomonads. Additionally, the functions of the type three secretion system (T3SS) in *P. cichorii*-plant interactions are not clearly clarified.
In this study, we investigated the role of the GacS-regulated lipopeptides and the T3SS in pathogenicity of *P.cichorii* SF1-54 on two host plants, chicory and lettuce, by deleting *gacS* and/or *hrpL*, which encodes the key sigma factor to control T3SS expression. Pathogenicity and phenotypic characterization of the wildtype and constructed strains were analyzed.
Compared with the wildtype, the *hrpL* mutant produced lipopeptides at a similar level but the *gacS* mutant was highly reduced in its lipopeptide production. The mutant deficient in *hrpL* did not significantly differ from the wildtype in induction of necrosis on chicory and lettuce, while the *gacS* mutant exhibited significantly less symptoms on both host plants compared to the wildtype and the *hrpL* mutant. Intriguingly, the *gacS hrpL*-double mutant no longer produced lipopeptides and lost pathogenicity on chicory but was still weakly virulent on lettuce. Thus, contribution of both the GacS-regulated lipopeptides and T3SS to pathogenicity/virulence of *P. cichorii* SF1-54 is highly associated with the hosts to be infected.

Keywords: *gac* regulon, cichopeptin, cichofactin, cyclic lipopeptides, *Lactuca sativa* L. var. *capitata*, midrib rot

Session 4
Poster 85

Toxin production by *Sarocladium oryzae*, the major sheath rot pathogen of rice

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Sheath rot is a very aggressive, emerging disease of rice. During the last decades, the disease has spread all over the world and has the power to destroy the total yield of a growing season. The major pathogen causing this disease is a fungus, called *Sarocladium oryzae*. This fungus produces at least two toxic compounds, helvolic acid and cerulenin. Helvolic acid is not only toxic for the plant, but also for other bacteria, while cerulenin is toxic for other fungi. According to the literature, exogenous application of both toxins can mimic sheath rot symptoms, but a clear correlation of these toxins with disease severity has not been demonstrated. The goal of this research is to study the diversity of *S. oryzae* isolates from Rwanda and their capacity to produce toxins. Therefore, we measure helvolic acid and cerulenin *in vitro* and *in planta* using LC-MS and study the genetic capacity to produce toxins using PCR with specific primers. Toxin levels were correlated with the pathogenicity of different *S. oryzae* isolates after rice inoculation using the grain inoculum technique. Results show that *S. oryzae* isolates differ tremendously in their toxin production. Ongoing research will further elucidate the role of these toxins in phytotoxicity.

Keywords: *Sarocladium oryzae,* helvolic acid, cerulenin, sheath rot

Session 4
Poster 86

Pathway-specific regulation of the botcinic acid biosynthetic gene cluster in the grey mould fungus *Botrytis cinerea*

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Botcinic acid (BOA) is a non-host specific phytotoxin produced by the polyphagous phytopathogenic fungus *Botrytis cinerea*. Its biosynthesis relies on the two PolyKetide Synthase encoding genes *Bcboa6* and *Bcboa9* which are clustered together with other co-regulated genes putatively involved in the pathway. In order to understand how BOA and its derivatives (botcinins) are regulated during the life cycle of *B. cinerea*, we investigated the genomic environment of the BOA cluster and searched for putative regulator encoding genes.
Amongst the clustered genes, *Bcboa13* was predicted to encode a $Zn(II)_2Cys_6$ transcription factor (TF). Fusion of Boa13 with GFP indicated that it localizes into nuclear foci. Inactivation of the *BcBoa13* gene resulted in a drastic diminution of the expression of the *Bcboa* genes, and in the absence of BOA and botcinins.
In addition to *Bcboa13*, another gene (*Bcboa1*) encodes a putative regulator: the predicted protein has a NmrA-like domain that may be involved in protein-protein interactions. The impact of BcBoa1 on botcinins production is investigated by gene inactivation, while its possible interaction with BcBoa13 is tested by Bimolecular Fluorescence Complementation (BiFC).
Finally, the BOA cluster is localized in a subtelomeric region in which the A+T/G+C-equilibrated regions that contain *Bcboa* genes alternate with A+T-rich regions (>85%) made of relics of transposable elements that have undergone repeat-induced point (RIP) mutations. The occurrence of RIP raises questions about possible chromatin-based regulation of BOA synthesis. Several chromatin modifiers (histone methyl transferases) are under studies to test this hypothesis.
Identification of BcBoa13 as the major regulator of BOA synthesis is the first step toward a comprehensive understanding of the regulation network of toxin synthesis in *B. cinerea.* Ongoing work may point out the respective role of pathway-specific transcriptional regulators and chromatin structure modifications.

Keywords: phytotoxin, secondary metabolism, $Zn(II)_2Cys_6$ transcription factor, NmrA-like protein, histone methyl transferases.

Session 4
Poster 87

Functional analysis of cassiicolin, effector of the rubber tree pathogen *Corynespora cassiicola*

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Rubber tree (*Hevea brasiliensis*) is the main source of natural rubber around the world. In Africa and Asia, it is affected by the Corynespora Leaf Fall disease caused by the necrotrophic fungus *Corynespora cassiicola*. Some strains of this pathogen secrete a small protein toxin, the cassiicolin, which play a role in the early stages of disease establishment.
In this study, we demonstrated the importance of cassiicoline for aggressiveness, by comparing the wild-type virulent CCP strain and the same strain deleted for the cassiicolin-encoding gene *Cas1* (CCPΔ*Cas1*), in interaction with rubber tree.
Deletion of *Cas1* gene did not modify major functions in the CCP strain: the growth rate, conidia production and percentage of germination were found similar for both strains. The wild-type and mutated strains were compared for their virulence on different rubber clones by analyzing the extent of symptoms induced after application of conidia suspension. Only hardly detectable lesions were observed with CCPΔ*Cas1,* suggesting that cassiicolin Cas1 is an important factor of virulence in rubber tree. Moreover, the two strains were compared for filtrate toxicity over a larger range of rubber clones, by measuring the electrolyte leakages induced on detached leaves by the application of purified cassiicolin Cas1 or culture filtrate from several *C. cassiicola* strains (including CCP and CCPΔ*Cas1*). The filtrate of the mutated strain was on average less aggressive than the wild-type filtrate although it still generated severe symptoms on a few clones, suggesting the involvement of toxicity factors other than cassiicolin. Hierarchical clustering (clones x treatments) based on these toxicity profiles grouped CCPΔ*Cas1* with strains of a different phylogenetic group (clade A) compared to CCP (clade C), without cassiicolin-encoding gene. These results demonstrate that, despite the production of other effectors by CCP, cassiicolin Cas1 is a major determinant of this strain controlling the virulence level in rubber tree.

Keywords: *Corynespora cassiicola*, rubber tree, cassiicolin, effector, deletion mutant

Session 4
Poster 88

Investigation of interactions between effector proteins of *Blumeria graminis* f.sp. *hordei* and barley resistance proteins

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The obligate biotroph powdery mildew fungus *Blumeria graminis* f.sp. *hordei* (*Bgh*) colonies barley (*Hordeum vulgare)* epiphytically. Supposedly for nutrient uptake and the secretion of effector proteins, the fungus establishes its feeding structure (haustorium) inside plant cells. To manipulate the host defense, the fungus is believed to use an arsenal of around 530 candidates for secreted effector proteins (CSEPs). Plants evolved with nonhost resistance (NHR) and R-gene mediated resistance two different layers of defense to prevent a successful colonization. Through former studies a number of barley genes have been identified that are associated with NHR. Some of these genes belong to the family of receptor-like-kinases (RLKs). R-gene mediated resistance relies on Nod-like receptors (NLR), which recognize specific effector proteins. Some of these NLRs evolved integrated decoy domains. These domains mimic target protein domains of effectors, possibly to attract them. Another potential target of effectors is the MLO protein, a negative regulator of defense to *Bgh*. The aim of this ERA-CAPS/DURES-Trit-funded PhD-Project is to analyze the physical interaction of selected barley RLKs with *Bgh* CSEPs. We also include a subset of barley decoy domains and the barley MLO protein to broaden the scope of this study. To discover protein-protein-interactions between CSEPs and RLKs, decoy domains and MLO proteins, we performed split-ubiquitin assays (a modified yeast two-hybrid method) with the barley proteins as baits. As preys we used a constructed CSEP library. The most meaningful interactions wil be tested by *in planta* bimolecular fluorescence complementation (BiFC) analysis and transient gene expression assays in barley single cells, which allow to determine the impact of these interactions on infection success. To identify amino acids/domains that are crucial for these interactions, we will use site-directed mutagenesis of bait and/or prey proteins. Our analysis will further improve our understanding of the barley-powdery mildew interaction.

Keywords: barley, powdery mildew, effectors, protein-protein interaction study

Session 4
Poster 89

Susceptibility of sweet corn genotypes on *Fusarium* spp. and biosynthesis their secondary metabolites

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At present we are observing considerable progress in sweet corn breeding, manifested in the considerable number of new cultivars of high economic value, differing in the length of the vegetation period, yield, contents of sugars and quality of kernels. With the appearance of new sweet corn hybrids, a need has been recognised to conduct research on their susceptibility to diseases caused by fungal pathogens, including *Fusarium* spp., mainly *F. proliferatum* and *F. verticillioides*, which synthesised toxic secondary metabolites like fumonisins, beauvericin or moniliformin.
The aim of the study was to estimate the susceptibility of various sweet corn genotypes (Sweetstar, Shine Rock, Overland, GSS 8529) under natural infection and after inoculation two isolates of *Fusarium* spp. separately - *Fusarium proliferatum* and *F. verticillioides* – representative for the population of these species in Poland, coming from the collection of pathogenic fungi PAS in Poznań.
In maize samples collected from three experimental groups (control - natural infection, inoculation with *F. verticillioides* and *F. proliferatum*) fumonisins from group B (FBs) – after extraction and purification – were analysed using HPLC methods.
In our studies, the differences between tested genotypes in mycotoxins levels were demonstrated. The highest FBs level shown for cv. GSS 8529 after inoculation both *F. verticillioides* (17.40 μg/g) and *F. proliferatum* (18.11 μg/g), whereas other genotypes were contaminated with fumonisins at similar lower levels of concentration (1.05-9.62 μg/g – inoculation with *F. verticillioides*, 2.47-6.64 μg/g – inoculation with *F. proliferatum*). Our results depended strongly on tested genotypes and isolates of *Fusarium* spp.
The part of this research was financially supported from the Polish National Science Centre project 2014/15/B/NZ9/02169

Keywords: beauvericin, fumonisins, *Fusarium proliferatum*, *F. verticillioides*, maize, moniliformin

Session 4
Poster 90

Autophagy in distant eukaryotic lineages is deregulated by an effector from the oomycete plant pathogen *Phytophthora parasitica*

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Oomycetes from the genus *Phytophthora* are plant pathogens, which have devastating impacts on agriculture and natural ecosystems. *Phytophthora parasitica* is a root pathogen with a hemibiotrophic lifecycle: during the initial stages of infection (biotrophy) the oomycete establishes an intimate contact with the living cells of the host, before inducing plant cell death to complete its life cycle (necrotrophy). cDNA libraries that were obtained from *P. parasitica*-infected tomato plants and onion epidermis cells display several sequences that encode secreted proteins with a canonical RxLR translocation signal, such as Avh195. Avh195 possesses three potential binding sites for ATG8, a key protein in the process of autophagy. Heterologous expression of Avh195 in tobacco plants slows down cell death responses such as those induced by proapoptotic BAX, and the HR inducers cryptogein and AvrPtoB. On this basis, we investigate the antagonism between death-inducing agents and Avh195 aiming at identifying the manipulated host signaling pathways, with a particular focus on the link between Avh195 and the autophagy machinery. To identify the molecular targets of Avh195, we initiated a trans-phylum analysis on plants, human cells, and green microalgae. Genetic expression of Avh195 dramatically alters the cellular phenotype in all these organisms, indicating that this protein targets an evolutionary-conserved mechanism.
*S. Testi is supported by the Labex “Signalife” [#ANR-11-LABX-0028-01].

Keywords: autophagy, *Chlamydomonas*, *Arabidopsis*, HeLa, oomycete, effector

Session 4
Poster 91

Transcriptomic and proteogenomic analysis of early gene expression profile of *Xanthomonas oryzae* pathovar *oryzae* in recognition of the host

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Genome-wide gene expression of *Xanthomonas oryzae* pathovar *oryzae* (Xoo) was quantitatively and time-resolvedly analysed in recognition of the host. The pathogenicity-related genes of the pathogen were activated using rice leaf homogenate. The gene expressions were quantitated with RNA-Seq and validated with qRT-PCR for seven different time points within 1 h post treatment. Global analysis of gene expression and regulation revealed the most dramatic changes in functional categories of genes related to inorganic ion transport and metabolism, and cell motility. Expression of many pathogenicity-related genes was induced within 15 min upon contact with RLX. *hrpG* and *hrpX* expression reached the maximum level within 10 and 15 min, respectively. Chemotaxis and flagella biosynthesis-related genes and cyclic-di-GMP controlling genes were downregulated for 10 min and were then upregulated. Genes related to inorganic ion uptake were upregulated within 5 min. We introduced a non-linear regression fit to generate continuous time-resolved gene expression levels and tested the essentiality of the transcriptionally upregulated genes by a pathogenicity assay of lesion length using single-gene knock-out Xoo strains. The in vitro system combined with RNA-Seq generated a genome-wide time-resolved pathogenic gene expression profile within 1 h of initial rice-Xoo interactions, demonstrating the expression order and interaction dependency of pathogenic genes. The synthesis and secretion of proteins followed late.

Keywords: Xanthomonas oryzae, time-resolved, gene expression, interaction

Session 4
Poster 92

Pathogenicity and genome characteristics of *Burkholderia* spp. causing rice grain rot in Korea

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Rice is an important cereal crop globally and the important rice diseases have been studied well. However, the climate change and cropping of restrict cultivars cause apprearance of new diseases. Therefore, it is necessary to conduct continuous disease monitoring. *Burkholderia* spp. are known as a pathogen causing rice grain rot and blight in seedling. This disease outbreak results in yield loss and a falling off in grain quality. Grain rot generally spread widely during the heading stage with hot and high humidity weather condition. As global warming progresses, the important of this disease is increasing.

From the discolored grain collected in the fields Korea, we screened 66 isolates by colony morphology and PCR-based screening. Then, 3 pathogens, *B. glumae* (GRbb 6)*, B. gladioli* (GRBB 15041, GRBB 15043)*, and B. plantarii* (GRBB 15061, GRBB 15061-1), were identified with 16S rRNA and phylogenetic marker genes sequencing. From phylogenetic analysis of 16S rRNA, these are difficulties in grouping between reference strain and Korean isolates. The isolates GRBB 15041 and GRBB 15043 were not assigned to cluster together with reference strain *B. gladioli*. These isolates were differentiated earlier than *B. glumae* and *B. plantarii* isolates, and formed a group. On the other hand, the isolates GRBB 15061 and GRBB 15061-1 were more near to *B. glumae* than *B. gladioli*. After sequencing analysis of *rpoD* and *gyrB* genes, these isolates were classified according to species. Therefore, to differentiate groups among *Burkholderia* spp. accurately, it is necessary to analysis other phylogenetic marker genes. As major factors showing pathogenicity against rice plant were detected as toxoflavin and tropolone using ultra-highperformance liquid chromatography equipped with mass/mass. The amount of these phytotoxins differed between the isolates according to their degree of pathogenicity. Results from this study, we revealed the relationship between Korean isolates, and evaluated their pathogenicity.

Keywords: *Burkholderia*, grain rot, rice

Session 4
Poster 93

Genome sequencing of isolates of the emerging pathogen *Ramularia collo-cygni* improves understanding of fungal lifestyle

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Ramularia Leaf Spot (RLS) has emerged to a major threat for barley production in many regions of the world. Due to the late appearance of unspecific symptoms there were especially molecular diagnostics that detected the fungus *Ramularia collo-cygni* (Rcc) throughout the lifecycle of barley and in samples worldwide as the biotic factor of the complex disease.
The economic impact of RLS is depending on the availability and effectiveness of control measures. Since there are no sufficiently resistant varieties, control is relying on the use of fungicides. In the past years *Rcc* has quickly adapted to the limited number of effective fungicide compounds.
The study of the biology of *Rcc* as a basis for sustainable control has been complicated by difficulties with traditional approaches by in vitro growth, sporulation, and inoculation.
To address urging questions, especially the uprise to a major disease, the relevance of seed transmission, and quick adaptation to control measures, the genome of *Rcc* (urug2 isolate) was de-novo sequenced. Fungal RNA from six different conditions was sequenced to support annotation and to uncover putative genes of interest. The assembled genome is about 32 Mb and the overall annotation enabled the prediction of 12346 genes. RNAseq data identified genes differentially expressed between the different conditions and enriched in the functional category “plant-pathogen interactions”. Many of those are homologues of genes expressed in planta by closely related *Zymoseptoria tritici*.
To evaluate the genetic diversity, whole genome sequencing of 19 *Rcc* isolates from multiple geographic locations and hosts was performed and mapped to the reference genome. Preliminary analysis indicated substantial genetic diversity and a possible population size expansion, which might explain the recent emergence of this fungus. The analysis is ongoing and recent conclusions on the pathogen biology will be presented.

Keywords: leaf spots, barley, *Ramularia collo-cygni*, genome, population genetics

Session 4
Abstract 94 (request for oral)

Multiple metabolites produced by *Pseudomonas* sp. CMR12a are involved in the suppression of *Pythium root rot* disease

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Root rot disease caused by *Pythium myriotylum* is the most devastating disease of the tropical tuber crop cocoyam (*Xanthosoma sagittifolium*) with production losses of up to 90%. This study was conducted in order to determine the role of phenazines (PCN), and two cyclic lipopeptides namely sessilins and orfamides, produced by the biocontrol strain *Pseudomonas* sp. CMR12a, in the suppression of the Cocoyam Root Rot Disease (CRRD). Previously generated biosynthesis mutants of CMR12a that were deficient in either one, two or all three metabolites were used in plant and microscopic experiments. Plant experiments revealed that mutants, which produced sessilins, orfamides or phenazines alone or at least any two of the metabolites, could effectively suppress the cocoyam root rot disease caused by *P. myriotylum* albeit in varying capacities. Microscopic analysis showed that 1 nM, 10 nM, and 25 nM of crude sessilins, purified orfamides, and phenazines, respectively, resulted in hyphal damage of *P. myriotylum* including vacuolization and lysis. Interestingly, during plant experiments, the null mutant, which did not produce any of the three metabolites, was able to give some level of disease suppression, indicating that CMR12a produces other compound(s) which could be antagonistic towards our target pathogen. Subsequent genome mining of the CMR12a draft genome revealed that a tabtoxin gene cluster is situated on the genomic island that harbors the phenazine-sessilin biosynthesis gene clusters. Furthermore, we show that a double deletion mutant which lost both the genomic island and the orfamide gene cluster could no longer suppress CRRD. In summary, our study reveals that multiple metabolites produced by *Pseudomonas* sp. CMR12a, can play independent and additive roles in the suppression of *Pythium*-mediated CRRD.

Keywords: *Pythium* spp, *Pseudomonas*, orfamides, sessilins, tabtoxin, cocoyam

Session 4
Poster 95

Antioxidant responses in 'Braeburn' and 'Golden Delicious' apple fruit to post-harvest storage disease caused by *Botrytis cinerea*.

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Apples are usually stored for a period of up to 6 - 9 months to ensure a steady, year-round supply of high-quality fruit. During this time, major losses may occur from post-harvest storage disorders and diseases. *Botrytis cinerea* is a post-harvest pathogen, which attacks over 200 different plant species, including apple. The present work examines the involvement of antioxidant metabolism of apple fruit in their susceptibility to artificial inoculation with *B. cinerea*. Apple fruit of two cultivars with contrasting susceptibility, 'Braeburn' and 'Golden Delicious', were inoculated on the sun-exposed and shaded sides. The antioxidant enzyme activity and antioxidant content of apple fruit were measured over time in peel and flesh samples taken from both sides of the fruit. Overall, 'Braeburn' was more susceptible than 'Golden Delicious' to *B. cinerea*, even though sun-exposed tissues of 'Braeburn' had higher initial levels of total vitamin C in peel and phenolic compounds in flesh, and higher of superoxide dismutase (SOD) activity and flavonoid peroxidase (POX) activity in all flesh tissues as compared to those of 'Golden Delicious'. In 'Braeburn', inoculation with *B. cinerea* provoked an antioxidant response, involving an increase superoxide dismutase (SOD) activity and ascorbate peroxidase (APX) activity, which were accompanied by the progressive oxidation of vitamin C, and a decrease POX activity. Disease tended to develop more rapidly on the shaded than on the sun-exposed side of 'Braeburn' fruit, involving a decrease of total phenolic content. Pre-harvest exposure to high light/high temperature stress is proposed to reduce the susceptibility of apples to subsequent postharvest pathogen. Disease susceptibility depended on different factors in the two cultivars. In 'Golden Delicious', vitamin C level remained low throughout, and antioxidant enzymes were not significantly induced as a result of inoculation. Thus, 'Golden Delicious' appears to utilize a different line of defence to fend off *B. cinerea.*

Keywords: *Malus* x *domestica*, postharvest storage, *Botrytis cinerea*, antioxidant metabolism

Session 4
Poster 96

Illuminating the role of extracellular vesicles in the interaction between microbes and plants

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Many microbes colonize the extracellular spaces between plant cells. A physical barrier is thus present between the two organisms. Yet, the signal exchange between plants and microbes over their cell surfaces remains poorly understood. Here we elucidate the role of microbe-derived extracellular vesicles, with their proteins, in the cell-to-cell communication with plants for the outcome of immunity. Our research focused on the bacterial model *Pseudomonas and the interaction with Arabidopsis thaliana*. We biochemically purified outer membrane vesicles (OMVs) of two *Pseudomonas* species and examined *A. thaliana* anti-bacterial immunity upon induction with OMVs. Surprisingly, plants were not protected against infection when pre-treated with OMVs from the bacterial pathogen *P. syringae pv tomato* DC3000 but exhibited increased susceptibility. However, Pto DC3000 OMVs are immunogenic inducing typical defence responses known for microbe-associated molecular patterns (MAMPs). To elucidate the mechanism by which pre-treatment with Pto DC3000 OMVs supports disease susceptibility, we determined their proteomic contents. Of these, 158 proteins were specific to Pto DC3000 OMVs while 351 proteins were found in both OMVs and the supernatant fractions. In agreement with the immunogenic activity of OMVs we found bacterial EF-Tu, a known MAMPs for A. thaliana, in the OMV proteome. Furthermore, the OMV proteome was enriched in a putative effector with a cellulase activity. The corresponding bacterial mutant in this cellulase was less efficient in binding Congo-Red, indicating a potential role in bacterial cell surface modifications. In summary, our results provide evidence of the involvement of OMVs from phytopathogenic bacteria to promote their virulence.

Keywords**:** outer membrane vesicles, plant immunity, MAMP

SESSION 5. FROM PLANT IMMUNITY TO INNOVATIVE PLANT BREEDING

Session 5
Poster 97

Elucidating the molecular and chemical responses of resistant rice to *Fusarium fujikuroi*

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The mechanisms of rice defense against *Fusarium fujikuroi* have not yet been fully clarified. In order to elucidate the factors involved in rice resistance against bakanae disease, an RNA-seq transcriptome study was performed. The molecular events that take place during the response of the resistant 'Selenio' cultivar and susceptible 'Dorella' cultivar were identified at 21 days post germination. The basic rice resistance machinery against *F. fujikuroi* involved PR genes, glucanases and peroxidases, since they were upregulated in both the resistant and susceptible cultivars. The specialized and evolved resistance mechanisms in the resistant cultivar included WRKY transcriptional factors, MAPK cascades, and some cytochrome P450 genes. These mechanisms were further confirmed by KEGG identification of Ca^{2+}-dependent protein kinase gene, JASMONATE ZIM-DOMAIN-like genes, *CEBiP*, *CERK1*, and *MYC2* genes, found only in 'Selenio'. These genes participate in one of the molecular patterns: response to chitin, jasmonic acid biosynthesis, and plant hypersensitive response. When the gibberellin production was controlled, the 'Selenio' plants activated the jasmonic acid metabolic pathway. In this way, 'Selenio' maintained its bakanae-resistance level. Increased concentrations of four rice phytoalexins were only found in 'Selenio'. The greatest increase in phytoalexin biosynthesis was observed for sakuranetin and momilactone A. The fungal pathogen in the resistant cultivar acts locally, at lower concentrations, and causes a rice hypersensitive response without any further damage to the plants.

Keywords: bakanae, jasmonic acid, *Oryza sativa*, phytoalexins, RNA-seq

Session 5
Poster 98

Monilia sp. infection weakens resistance of transgenic plum *Prunus domestica* L., cv. HoneySweet to the *plum pox virus*

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Sharka caused by *Plum pox virus* (PPV) is the most harmful disease of stone fruits in Europe and elsewhere in the world. There is no highly PPV resistant cultivar of plum. Biotech approach has led to the development of resistance through genetic engineering. In this study, we evaluated a transgenic plum *Prunus domestica* L., clone C5 (cv. HoneySweet), where the PPV resistance is based on RNA interference (RNAi). Resistance in C5 plums has been evaluated for PPV, *Prune dwarf virus* (PDV), and *Apple chlorotic leaf spot virus* (ACLSV) in a regulated field trial in the Czech Republic for fifteen years (2002-2016). No natural infection of PPV by aphids was recorded in C5 plums. Co-infections of PPV with PDV and/or ACLSV had practically no influence on stability of resistance to PPV in C5 trees. Even under high and permanent infection pressure introduced through graft inoculation of the viruses, PPV has been detected in C5 trees only in several leaves situated close to the point of inoculum grafting in the first nine years. Mild symptoms of PPV disappeared year by year. No PPV symptoms were observed in the following three years (2011-2013) and results of ELISA detection tests were negative. Similar results were obtained, when RT-PCR was used for PPV detection. There was a severe attack of transgenic plum trees by *Monilia* sp. in the twelfth year. Mild PPV symptoms have appeared again in several leaves in the next two years (2014-2015) after the *Monilia* sp. infection and disappeared in the fiteenth year. The presence of PPV was confirmed by ELISA and RT-PCR not only in symptomatic leaves, but also in several fruits showing no symptoms. The low presence of PPV was confirmed in several asymptomatic leaves and fruits in the fifteenth year of evaluation, too.

Keywords: Plum pox virus, resistance, transgenic plum, cv. HoneySweet, *Monilia* sp., infection, resistance weakening

Session 5
Poster 99

Development of a mycorrhization method of grapevine in semi-hydroponics as a tool for studies of defense responses against pathogens

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Mycorrhization by arbuscular mycorrhizal fungi (AMF) has been demonstrated to have positive effects on plant growth and resistance to many biotic aggressors (Pozo & Azcon-Aguilar, 2007) and could be an interesting way to reduce or replace use of pesticides.
Studies of AMF impact on grapevine defense responses during pathogen challenge would be facilitated by a procedure to obtain rapidly a large number of mycorrhizal plants in controlled conditions of nutrition and environment. Access to the root system should be also simplified to check the presence of AM fungus and/or soil-borne pathogens after inoculation.
For this purpose, we developed a method to obtain mycorrhizal grapevine plants acclimatized from *in vitro* culture in semi-hydroponics.
Six-old-weeks *in vitro* plantlets of the rootstock 41B were transplanted in individual pots containing 1:1 sterilized sand/vermiculite and acclimatized in growth chamber (25°C/20°C day/night, 16h photoperiod) during five weeks. They were then inoculated with an AMF strain of *Funneliformis mosseae* spore suspension. Mycorrhization level was checked six weeks later according to the method of Trouvelot et al (1986).
Root system of plants was mycorrhized with a frequency comprised between 90 and 100% and an intensity of 51.3-69.8%. Proportion of arbuscules was from 70 to 95% in mycorrhizal parts and from 44 to 60% in whole root system. AM fungus root colonization is thus particularly efficient in this system comparing to others results reported in literature. For instance, Eftekhari et al (2012) reported mycorrhizal frequency of 70% for this AMF species in grapevine plants from hard-wood cuttings.
With this method a lot of homogeneous highly mycorrhizal grapevine plants can be obtained rapidly, all over the year, in standardized growing conditions and without growth inhibition. This tool will be used for analysis of grapevine defense reactions against aboveground or soil-borne pathogens.

Keywords: grapevine, mycorrhization, arbuscular mycorrhizal fungi, semi-hydroponic culture, acclimatized *in vitro* plants

Session 5
Poster 100

Study of a soybean inducible promoter by *Phakopsora pachyrhizi*

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Phakopsora pachyrhizi is a biotrophic fungus responsible of the Asian Soybean Rust (ASR), a disease causing important yield losses in soybean producing countries. It has been shown that *P. pachyrhizi* infection triggers important transcriptional changes in soybean (Tremblay *et al*., 2010 and 2011) with more than hundreds of genes up or down regulated as a consequence of promoter modulation. Study of the response of promoters to pathogens is central to understand the gene regulation in plant which is important for biotechnological applications. In this work we focused on the identification and characterization of a soybean promoter induced by *P. pachyrhizi*. Analyze of internal and external RNAseq data allowed to select a chitinase gene strongly up-regulated at 24h post infection for 10 days, but not by chitin treatment. We first validated and investigated the induction of this promoter during *P. pachyrhizi* infection by qRT-PCR. Then stable soybean lines expressing a GFP under the control of this promoter were generated. GFP fluorescence was quantified (+/-) after biotic and abiotic stresses to determine the promoter specificity. We also looked at the activity of this promoter after activation of different signaling pathways implicated in plant defense response. The results showed that this promoter induced by *P. pachyrhizi*, was not induced by a necrotrophic fungus and physical wounding. Activation of Jasmonate, Ethylene and Salicilate signaling did not trigger induction of this promoter. We demonstrated that this promoter could be specifically induced by *P. pachyrhizi* to the contrary of other pathogen-inducible promoters often activated by several stimuli.

Keywords: soybean, *Phakopsora pachyrhizi*, induction, promoter, GFP fluorescence

Session 5
Poster 101

Resistance to fusarium ear rot in maize: heritability and trait associations

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Red and pink ear rots caused by Fusarium spp. are important factors affecting the yield and its quality, mainly because of contamination with mycotoxins produced by the fungi. The development of resistant host genotypes strongly depends on availability of sources of resistance and information on host pathogen interactions. The mode of inheritance of resistance appears to differ: additive, possibly non-additive effects, digenic and polygenic patterns have been identified. It depends on several components such as resistance to initial infection, resistance to fungal degradation of silk tissues, resistance to fungal spread by through a wax layer in the grain or grain morphology and chemical compounds of the pericarp. The accumulation of toxins can also be affected by the plant genotype. Although selection is effective to reduce disease severity after inoculation with *F. graminearum*, additional genes seemed to affect grain DON concentration (i.e., ratios DON/DS in grains depended on genotype), indicating that specific mechanisms are present in the plant affecting DON production by de fungus and additional genetic progress would be achieved by including grain DON concentration as a selection parameter. The present research was conducted to estimate heterosis, heritability and correlation coefficients to ear rot in set of F1 crosses generated from resistant and susceptible parents. The positive heterosis for ear rot resistance and DON content was observed. When the two parents components were susceptible or highly susceptible the heterosis effect for disease symptoms was more than 36% and for DON more than 86%. Additionally, narrow-sense heritabilities h2ns for ear rot and DON content were very high – depend of combination more than 90%. This work was funded by Polish Ministry of Agriculture and Rural Development Proj. No. 4-1-06-3-01 (33).

Keywords: maize, ear rot, heterosis, heritability

Session 5
Poster 102

Effective resistance to powdery mildew (*Blumeria graminis* f. sp. *hordei*) in winter barley in Poland

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The presented investigation describes the introduction of the mlo gene for resistance to powdery mildew (B. graminis f.sp. hordei) into winter barley cultivars using MAS strategy. These cultivars are characterized by high and stable yield under Polish conditions. Field testing of the obtained lines with Mlo resistance for their agricultural value was conducted. Four cultivars (Souleyka, Titus, SU Vireni and Metaxa) as high yielding parents were used. In addition, existing resistance genes to powdery mildew in these cultivars were preserved. Two lines (BKH 735 and line 42) as parents with Mlo resistance were used. Line BKH 735 was obtained in IHAR-PIB Radzików in 2002-2011. Selection for presence of the mlo gene was conducted in backcross populations by phenotyping in the field (natural infection) and under greenhouse conditions (differential barley lines for resistance genes for powdery mildew and differential fungus isolates). In addition, to confirm the presence of the mlo gene in backcross populations MAS strategy was applied using SSR markers HVmlo1 and HVmlo3. Field trials with backcrossed lines were conducted during 2016/17 in 3 locations: in Central (Radzików) and Western Poland (Szelejewo, Wiatrowo). The parental lines were used as control. The aim of these trials was to obtain information on agricultural value of obtained lines. Our results demonstrate the practical use of the introduction of Mlo resistance into background of winter barley germplasm with valuable economical characteristics in Polish agricultural conditions. This work was conducted in the project: Interaction between powdery mildew (Blumeria graminis f.sp. hordei) resistance determined by mlo gene and economical value characteristics in winter barley. 2014-2020. Prog.: Basic Research for Biological Progress in Crop Production; Funded by the Ministry of Agriculture and Rural Development Proj. No. 41-04-3-01 (27).

Keywords: winter barley, pre-breeding, powdery mildew, Mlo resistance, MAS, SSR markers

Session 5
Poster 103

Nitrogen limitation moderately affects plant response to biotic stress

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In order to determine how defense activation is affected by mild nutrient limitation, we performed a microarray analysis. Plants were grown in full or limiting nitrogen and subjected to biotic stress. Firstly, the effect of abiotic stress (N limitation) was observed on global gene expression patterns in response to biotic stress (bacteria). In response to the biotic stress, we found that N limitation had a mild impact on the set of genes modulated in response to infection although the limitation had a visible effect on plant growth. However, some related-defense genes were modulated by plant N status. We then considered that Arabidopsis plants were subjected to two individual abiotic and biotic stress treatments "N limitation" and "bacteria" respectively, as well as a combination of both. We further presented and analyzed the homogeneous dataset of plant responses to single and combined stresses according to Rasmussen *et al,* 2013. When two individual stresses were combined, 33 % of transcripts responded in non-predictable manner. Only a small fraction (3 %) of the transcripts prioritized between potentially antagonistic responses. Moreover, several known defence-related genes were regulated in the prioritized mode reflecting an alteration in plant defense program under N limitation treatment. Analyze the microarray data indicated that Arabidopsis genes were regulated to a greater extent by bacteria than N limitation, suggesting a dominance in a pair of abiotic and biotic stress treatment in terms of genes modulation.

Keywords: abiotic, biotic, defense, plant, stress

Session 5
Poster 104

Promising sources of resistance to pathogens of winter wheat in Ukraine

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In the laboratory of immunity of agricultural crops to diseases of NAAS Institute of Plant Protection was developed the technology of creation of artificial complex infectious background (ACIB) pathogens *Puccinia recondita* f. sp.tritici Rob. et Desm., *Septoria tritici* Rob. et Desm. and *Pseudocercosporella herpotrichoides* (Fron.) Deighton on provocative background of *Blumeria graminis* DC Speer sp. tritici E.M. Marchal on winter wheat in the field. There synthetic population of pathogens, based on the annual surveys of winter wheat crops in different regions of Ukraine and data on the racial composition and internal population structure of powdery mildew pathogens, brown rust, septoria leaf blotch, eyespot pathogen, was formed. There were evaluated the resistance of 34 varieties of winter wheat from different ecological and geographical origin during 2014-2016 in Forest-Steppe zone of Ukraine using ACIB and separate artificial background of infectious pathogen *Tilletia caries* (DC) Tul.

We have allocated sources of resistance to powdery mildew: Vluchna, L168-27, L155-03KH, Zolotoverha, Benefis, Niva, Hvulya (Ukrainian selection), MV Laura (HUN), Shahriar (IRN), Nikifor (ROU); to root rot – Sofia Kyivs'ka (UKR), Mukhran (GEO), MV 17 / Zrn (IRN). The systemic resistance against pathogens of powdery mildew and bunt had next varieties Syaivo (UKR), Miranda (ROU), F94578G3-1 / BUCUR // DELABRAD (ROU). Variety Midas (AUT) showed resistance to powdery mildew and root rot. Varieties with resistance to septoria leaf blotch and brown leaf rust were not found.

Search effective sources of resistance using artificial backgrounds of infectious pathogens enables breeders get promising starting material for the creation of new productive varieties, which can overcome of the infection pathogens.

Keywords: artificial complex infectious background, pathogen, sources of resistance, winter wheat

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Poster 105

Comparative analysis of resistance of oats genotypes to contamination by different *Fusarium* species and mycotoxins

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The aim of our study was to evaluate the amount of DNA and mycotoxin content of 24 oats cultivars after their artificial inoculation with *Fusarium culmorum* and *F. sporotrichioides* separately.
The characteristics were based on sum of two features: the DNA content of trichotecenes producing *Fusarium* fungi (Tri-*Fusarium*) and the amount of mycotoxins in grain of cultivars: deoxynivalenol (DON) or T-2 toxin.
The genotypes inoculated with aggressive *F. culmorum* strains contained DNA of Tri-*Fusarium* from 0.03 to 3.64 pg/g of milled grain, the amount of DON ranged from 20 to 1179 ppb. The quantity of DNA of Tri-*Fusarium* in the samples inoculated by *F. sporotrichioides* strains varied from 0.03 to 1.93 pg/g, the amount of T-2 toxin ranged from 0 to 133 ppb. The high positive correlation between the amounts of Tri-*Fusarium* DNA and DON was observed ($r=+0.84$, $p<0.001$), while the strong correlation between the Tri-*Fusarium* DNA and T-2 toxin amounts was not found.
The group of relatively resistant genotypes to *Fusarium* infection (both *Fusarium* pathogens) included cultivars Bisuandorodu (Russia), Geszty (Hungary) and Gehl (Canada). The group of high susceptible cultivars consisted of Medved and KSI 432/08 (Russia), Bessin and Hurdal (Norway). Relationship between DNA contents of Tri-*Fusarium* in the grain of genotypes inoculated by two species was relatively weak ($r=+0.21$). The most reliable results were obtained under inoculation of plant genotypes by the most aggressive pathogen.
A breeding strategy to combine the resistances to pathogens and mycotoxins accumulation would probably lead to the development of new oats varieties, which able to efficiently limit the problem of mycotoxin in grain.
The investigation was supported by the Russian Science Foundation (No. 14-16-00072).

Keywords: *Avena*, genotypes, fungi, *Fusarium*, real-time PCR, mycotoxins

Session 5
Poster 106

Combined transcriptome and metabolome analyses to understand the response of flax (*Linum usitatissimum*) to the pathogenic fungus *Verticillium dahliae*

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Verticillium dahliae is a major flax pathogen (*Linum usitatissimum*). The current increase of its frequency leads to significant economic loss in flax cultivation. This plant disease remains misunderstood and the versatility of the pathogen as well as the lack of flax resistant cultivars make its propagation difficult to control.
This study consists in identifying transcriptomic and metabolomic characteristics in plants displaying various behaviors towards the disease in order to develop markers for marker-assisted breeding.
An artificial infection protocol was optimized under controlled conditions. Using this system, several flax cultivars were screened, allowing identification of two cultivars, one partially resistant (Évea) and one susceptible (Violin), which were subsequently used as models in this study.
The pathogen progression in these two flax cultivars was studied using qPCR quantification and a correlation was found between the amount of pathogen in plants and the severity of symptoms. These results showed that during their growth, despite a rapid colonization of the whole plant, tolerant flax cultivars were able to maintain a lower amount of fungi within theirs tissues.
Transcriptomic and metabolomic analyses were performed on infected and non-infected plants from the two selected cultivars using microarrays, RT-qPCR and NMR techniques. For each cultivar, RNA and metabolites were extracted at different stages of development between seven days and three weeks after infection. Microarrays data showed a clear response in the most resistant cultivar while no significant change was observed in the susceptible one. This response was characterized by expression changes for genes involved in reception and transduction of pathogen signals, hormone signaling, and activation of pathogenesis-related, chitinase and β-1,3-glucanase biosynthesis. NMR analyses revealed that the pathogen affects the metabolome of the two flax cultivars, in particular in amino acids, sugars and polyamines contents.

Keywords: flax, *Verticillium*, transcriptome, metabolome, microarrays, NMR

Session 5
Poster 107

"Omics-" for a mapping of grapevine response to elicitors and identification of induced resistance markers

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Most cultivated grapevine cultivars (Vitis vinifera cvs) are susceptible to severe cryptogamic diseases such as downy mildew caused by the obligate biotrophic oomycete Plasmopara viticola. Fungicides are usually employed to control these diseases. However, in a context of sustainable viticulture, complementary and / or alternative strategies, such as elicitor-induced resistance, are expected. Despite numerous elicitors from diverse origins have been identified, their mode of action and impact on plant metabolism remain often largely unknown. We therefore used a multiple analyses approach encompassing Proteomics, Metabolomics, Transcriptomics and Volatilomics to generate and cross-compare data to gain insight into the mode of action of elicitors (impact on the plant metabolic pathways) and to uncover putative markers of induced resistance (IR).
Methods : Sulfated laminarin (PS3), a sulfated b1,3-glucan affording a high level of grape downy mildew control (90%) in greenhouse conditions, was the main elicitor used for this studies. Elicitor-treated leaves were collected and extracts were prepared for transcriptomic (using the Roche-Nimbelgen Grape array), proteomic (2DE separation and nanoLC-MS/MS analysis) and metabolomic (FT-ICR-MS) analyses. For volatilomic, Volatile Organic Compounds (VOCs) were caught onto SPME fibers prior to GC-MS analysis.
Results : These different -omics approaches allowed us to point out the main metabolic pathways involved in PS3-IR and to identify possible markers of PS3-IR. Among them were PR10, farnesene, and erythritol phosphate.
Conclusion : These global omics- analyses provided large amounts of valuable data that enrich our knowledge of elicitor-IR. Moreover, they allowed the identification of IR-markers that would be useful tools for further screening of putative elicitors and to follow the plant response to elicitor treatment in field conditions.

Keywords: -omics, elicitor, induced resistance, grapevine

Session 5
Poster 108

The effectiveness of translocation 1AL/1RS in resistance to leaf rust in Ukraine

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In 2011-2015 the effects of translocation 1AL/1RS on resistance to the natural population of leaf rust races, was investigated. It is spent an assessment of resistance of carriers of translocation 1AL/1RS (12 cultivars - Amigo, TAM107, TAM201, Nekota, Century the American selection and domestic selection - Monologue, Colombia, Zolotokolosa, Eritrospermum 26221, Knyaginya Ol'ga, Smuglyanka, Spasivka) at a various infectious load. In 2011 and 2015 observed average level of disease development. In 2014 - high infection level, and in 2012 and 2013 recorded epidemics.

Stable resistance to local population of leaf rust were cvs Necota, Century, Monologue,Smuglyanka, Spasivka. At recorded epidemics they showed high resistance. It is known, that in these cultivars contain resistant genes Lr24, and at cv Century also Lr42. Considering their high stable resistance it is not the whole set of genes of resistance. Cvs Amigo and Colombia have shown variable resistance (decrease in a year of epidemics, followed by restoration with at reducing of infection level). Other cvs Zolotokolosa and Eritrospermum 26221 under epiphytotic 2013 lost stability, but regained it when reducing of infection load in 2014 and 2015. The cv Knyaginya Ol'ga for all years of studies showed the resistance - moderate susceptibility. It contains gene Lr34, which in the conditions of Ukraine are not effective. Lines TAM107, TAM201 are resistant only in years with an average infectious level of development. When epiphytotics they are susceptible, and in the subsequent year are moderate susceptible.

Thus, exhibiting of resistance of carriers of wheat-rye translocation 1AL/1RS in the conditions of Ukraine variously. In case of epiphytotic disease, it does not fully provide resistance of cultivars. The presence background of translocation 1AL/1RS other resistant genes increases the resistance of cultivars. Such features must be considered when attracting of carriers of translocation 1AL/1RS in breeding process.

Keywords: winter wheat, translocation 1AL/1RS, leaf rust, resistance, genes

Session 5
Poster 109

SWEET » sugar transporters in grapevine: role in the interactions with bioaggressors

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Grapevine is susceptible to diverse pathogens including oomycetes, fungi, bacteria and viruses. Carbon allocation and sugar partitioning play key roles in plant-bioagressor interactions. There is a competition for sugar at the plant-pathogen interface which is controlled by membrane sugar transporters as the SWEET transporters (Chen et al. 2010). These proteins are a target of extracellular pathogens which modify their expression to acquire the necessary sugars to their growth (Chu et al. 2006). The aim of this study is to characterize the function of grapevine SWEET transporters in the interaction with pathogens with different lifestyles.

In previous work, we characterized the SWEET family of sugar transporters in *Vitis vinifera*, and showed that VvSWEET4 could be involved in plant cell death and resistance to *Botrytis cinerea* (Chong et al. 2014). We also identified two additional grapevine SWEET transporters up-regulated after inoculation with *Plasmopara viticola* and *Erysiphe necator*. To assess the sugar transport specificity of *VvSWEET4*, the gene was overexpressed in grapevine hairy roots. Our results show that hairy roots overexpressing *VvSWEET4* have a higher growth than controls on sucrose and glucose-containing media. Transport experiments with radiolabeled sugars will confirm the transport activity of VvSWEET4.

We obtained different knockout mutants of *AtSWEET2* and *AtSWEET17* and overexpressors of *VvSWEET2a and VvSWEET17d* genes in *Arabidopsis*. We studied the susceptibility levels of these lines to the bacteria *Pseudomonas syringae* pv. *tomato* and the oomycete *Hyaloperonospora arabidopsidis*. Preliminary results suggest an effect of *AtSWEET2* on *P. syringae* infection.

To validate our results in grapevine, we are creating plants where our candidate genes are either overexpressed or knocked-down. This material will help to elucidate the involvement of grapevine SWEET transporters in the interaction with pathogens and provide information concerning sugar transport and partitioning in those interactions. Finally, the involvement of grapevine *SWEET* genes in plant-pathogen interaction could lead to the identification of new recessive resistance genes.

Keywords: grapevine, SWEET transporter, sugar transport, pathogen resistance

Session 5
Poster 110

Traditional and Modern Plant Breeding Methods with Examples in wheat

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The aim of presented research is pyramiding of leaf rust and powdery mildew resistance genes in a one genotype. As the donor of resistance to leaf rust several lines were used. The line KS90WGRC10, which carries the *Lr39* (= *Lr41*) gene derived from the diploid wild wheat *Triticum tauschii* (syn. *Aegilops squarrosa*). The line KS04WGRC45, that carries the *Lr55* gene derived from the *Elymus trachycaulus*. The line HRS Yecora Rojo, which carries the *Lr47* gene derived from *Triticum speltoides*. As the donor of resistance to powdery mildew two lines were applied. A 6VS/6AL translocation line of Yangmai5 that carries the *Pm21* gene derived from the wild cv. *Dasypyrum villosum*. Line NC99BGTAG11, which carries the *Pm37* gene derived from the *Triticum tiomopheevii* and line NC97BGTD7, which carries the *Pm34* gene transferred from the *Aegilops tauschii.* To detect resistance genes (foreground selection) several molecular markers for *Lr41*, *Lr47*, *Lr55*, *Pm21* and *Pm37* were applied. In addition, plant materials were inoculated in the greenhouse at the three-leaf stage with a natural pathogen population of *P. recondita* and *B. graminis.*
The marker-assisted selection and resistance tests allowed to obtain homozygous lines carrying three or four resistance genes to leaf rust and powdery mildew in winter wheat. Moreover, the newly produced homozygous wheat lines, can be used as the source of effective resistance for the leaf rust and powdery mildew by geneticists, breeders and plant pathologists.

Keywords: *Blumeria graminis*, gene pyramiding, marker-assisted selection, *Puccinia recondita*

Session 5
Poster 111

Identification of candidate genes involved in innate immunity and effector-triggered defence

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The innate immune system of plants consists of two layers. The first layer of defence relies on recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs). Host-adapted pathogens secrete effector proteins to dampen or evade host PAMP-triggered immunity (PTI). Resistance (*R*) genes encode proteins that are involved in recognition of effectors or their host targets to activate effector-triggered immunity (ETI) or defence (ETD) against intracellular or extracellular fungal pathogens. Whereas *R* genes that encode cytosolic receptors are well characterised, those that encode transmembrane receptors are not. We discuss the use of advanced computational methods to better define the *R* gene complement in genomes of plants and to assist the identification of genes involved in PTI that may contribute to partial or quantitative disease resistance. These tools can be an asset for plant breeding and crop protection.

Keywords: computational biology, disease resistance, machine learning

Session 5
Poster 112

Evaluation of selected cultivars *Cucurbita maxima* type Uchiki Kuri for resistance to *Zucchini yellow mosaic virus*

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Zucchini yellow mosaic virus (ZYMV) causes considerable losses of cucurbitaceous vegetables grown nearly all over the world. Commonly planted cultivars are highly susceptible to ZYMV especially orange cultivars of pumpkins *Cucurbita maxima* type Uchiki Kuri. Fruits collected from infected plants are often dark-green stained and their shelf life is rapidly shortened. Therefore looking for resistant cultivars *Cucurbita maxima* has started. Three cultivars from South Africa, three from the Czech Republic and two from the Netherlands were selected for evaluation their resistance to the most virulent Czech ZYMV-H strain (GenBank Acc. No. DQ144054). Zucchini squash (*Cucurbita pepo*) 'Zelena' **was added to this group** as an internal standard of a susceptible cultivar to ZYMV. Tested plants were sown in a greenhouse and coming up plants were mechanically inoculated by ZYMV-H. Their resistance was assessed by comparison of the virus protein relative concentration and observed symptoms four weeks after the innoculation. The relative concentration of ZYMV protein in leaves was calculated from the virus titer determined by ELISA. The South African pumpkins 'Invincible' and 'Star' were evaluated as highly resistant; the virus concentration in their leaves was nearly zero and they did not show any viral symptom on their leaves and fruits but they were ZYMV positive in PCR. The Dutch recent hybrids E 30R.080 F1, E 30R.079 F1 and South African 'Baby pumpkin' were medium resistant; the virus multiplied in these plants in a low rate. Czech pumpkins 'Blue Kuri', 'Grey Queen' and 'Hokkaido' were evaluated as highly susceptible; the virus concentration in plants was the same or higher than in the internal susceptible standard 'Zelená' and their leaves and fruits were severely damaged by ZYMV infection. In conclusion, pumpkins 'Invincible' and 'Star' are appropriate for planting in ZYMV infected areas and we can recommend them for breeding new resistant cultivars.

Keywords: virus concentration, virus titer, ELISA, PCR, ZYMV

Session 5
Poster 113

Pharmacological screen for suppressors of *mlo*-mediated powdery mildew resistance

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Powdery mildew resistance conferred by absence of *MLO* gene featured with broad spectrum penetration resistance. The molecular mechanism underlying this resistance is still elusive. We try to identify new molecular components involved in *mlo*-mediated resistance via pharmacological approach. We screened 65 chemicals with viarant molecular targets by peeling method in barley-*Blumeria graminis f. Sp. Hordei* interaction system, out of which twelve chemicals were able to induce susceptibility of barley *mlo* mutant according to results of penetration counting. Targets of the positive chemicals include cAMP biosynthesis and vesticle trafficking, and targets of variant polyamine species and alloxan which showed positive effect are not yet identified.

Keywords: *MLO* gene, pharmacological screen, barley, powdery mildew, cAMP biosynthesis, vesicle trafficking

Session 5
Poster 114

Investigating induction of SAR during gene-for-gene interactions between *Arabidopsis thaliana* and *Pseudomonas syringae*

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Plants deploy two key active defensive strategies to combat microbial pathogens; (i) Recognition of Pathogen-Associated Molecular Patterns (PAMPs) by extracellular surface receptors leading to the activation of PAMP-Triggered Immunity (PTI); (ii) Recognition of pathogen effector activity, usually intracellularly, by host Resistance (R) proteins leading to Effector-Triggered Immunity (ETI). ETI is characterised by a rapid localised Hypersensitive Response (HR). HR induces Systemic Acquired Resistance (SAR) through the production of an inducible immune signal(s), leading to broad spectrum systemic resistance. We investigated the earliest events associated with SAR signalling using plant electrophysiology, SAR mutants and a unique promoter-luciferase fusion that captures early systemic transcriptional events underlying initiation of systemic immune signal(s). We describe the transcriptional dynamics of *A70* (At5g56980), a gene of unknown function (Truman *et al.* 2007), in local and systemic tissue following challenge with different elicitors and virulent or avirulent pathogen challenges. We provide evidence that *A70* responds to a jasmonate (JA) related signal that is rapidly generated following ETI recognition. We further evaluate *A70::LUC* reporter activity in response to JA stimulus and correlate activity with histological expression of a JA repressor reporter (*JAZ10::GUS*) and *A70::GFP* reporter in systemically responding leaves following avirulent pathogen challenges. Finally, we examine changes in electrophysiological signals following ETI in local and systemic leaves. Focussing on events underpinning initiation, propagation and perception of SAR-inducing signals within the first 6-8 h of pathogen challenge we provide new insight into the integrated signalling mechanisms, dynamics and connectivity underpinning systemic immune responses. We conclude that their multicomponent signals that link ETI induced transcriptional and electrical signals, with a COI1 receptor propagative transcriptional wave the leads to rapid temporal spatial transcriptional activation of jasmonate responsive genes in systemic responding leaves.

Session 5
Poster 115

Next generation cross protection: single virus with multiple targets

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Plant viruses are obligate intracellular parasites that infect many agriculturally important crops and cause severe losses each year. Genetic resistance is considered the most effective means to control these viruses. However, extensive screening of germplasms for natural genetic resistance only identifies a few resistance genes. Cross-protection is the practice of protection of plants against a viral infection by prior inoculation of them with a mild version of the virus. This approach has been used to control many important viral diseases such as *Citrus tristeza virus* (CTV), *Cucumber green mottle mosaic virus* (CGMMV) and *Pepino mosaic virus* (PepMV) in the world. The possible mechanisms underlying cross-protection include RNA silencing and superinfection exclusion. This raises the possibility that an attenuated virus (serving as a viral vector) may be modified to contain genomic fragments of different viruses and of a host factor gene of multiple viruses. Thus, this modified virus may be used for cross-protection against several viruses. Here, we present our data on the development of a viral vector derived from *Prunus necrotic ringspot virus* (PNRSV), a widespread fruit tree virus that is endemic in all *Prunus* fruit production countries and regions in the world. The modified PNRSV vector, harboring the sense-orientated target gene sequence of 100~200-bp in length in genomic RNA3, could efficiently trigger the silencing of a transgene or an endogenous gene in the model plant *N. benthamiana*. The PNRSV-based vector could also be manipulated to silence endogenous genes in peach such as eukaryotic translation initiation factor 4E isoform (eIF(iso)4E), a host factor of many potyviruses including *Plum pox virus* (PPV). Moreover, the *eIF(iso)4E*-knocked-down peach plants were resistant to PPV. Our data open a potential avenue for the control of virus diseases in perennial trees via viral vector-mediated silencing of host factors.

Session 5
Poster 116

***Burkholderia phytofirmans* PsJN confers grapevine resistance against *Botrytis cinerea via* a direct antimicrobial effect combined with a better resource mobilization**

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Plant growth-promoting rhizobacteria (PGPR) are of great interest since they are beneficial naturally occurring soil bacteria that colonize plant roots and confer beneficial effects. They can increase yield, stimulate plant growth, reduce pathogen infection, and reduce biotic and abiotic stresses. Among these PGPRs, endophytes are defined as those bacteria that are able to colonize the internal tissue of the plant without causing external signs of infection or negative effects on their host. *Burkholderia phytofirmans* PsJN, classified as an ePGPR, was first isolated from surface-sterilized onion roots infected with the mycorrhizal fungus *Glomus vesiculiferum*. This rhizobacterium significantly promotes growth and protects the grapevine against biotic (grey mould disease) and abiotic (cold) stresses. If mechanisms implied in cold tolerance induced by PsJN were elucidated, the protective effect induced by the PGPR against B. cinerea however remains elusive. To unravel the mechanistic of pathways involved in the observed resistance, different traits of the tripartite interaction between *Vitis vinifera* L., *Botrytis cinerea* and *Burkholderia phytofirmans* were highlighted. Among these aspects, direct antimicrobial action of PsJN, the ability of the bacterium to prime defense responses and carbohydrate metabolism of grapevine will be presented.

Keywords: grapevine, *Paraburkholderia phytofirmans* PsJN, *Botrytis cinerea*, defense, priming

SESSION 6. EMERGING TOOLS FOR THE MANAGEMENT OF PLANT DISEASES: AGROECOLOGY AND DISEASE MANAGEMENT

Session 6
Poster 117

Improvement of Norwegian potato production through better seed potato health

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Potatoes are the most important food crop with respect to first hand value in Norway. Particularly challenging is the fact that many of the important potato pathogens can be difficult to detect as they give rise to latent infections. A first step towards management of plant diseases is appropriate identification and detection methods. Consequently, one of the major goals of this study was to implement qPCR for rapid and sensitive detection and quantification of latent infection of bacteria and fungi in seed tubers. The work was focused on bacterial soft rot diseases *(Pectobacterium* spp. and *Dickeya* spp.), late blight (*Phytophthora infestans*), gangrene (*Boeremia foveata*) and skin blemish diseases such as silver scurf (*Helminthosporium solani*), skin spot (*Polyscytalum pustulans*) and black dot (*Colletotrichum coccodes*). During the first two years of the project, qPCR testing for the abovementioned pathogens were implemented and 15 seed lots were tested each year. Samples from the seed lots were tested at three time points, before planting, after harvest and after three months in storage. Skin spot was found in all seed lots except from the pre-basic seed lots. Gangrene could be detected in all lots, while low levels of late blight was found in a quarter of the seed lots. Low levels of black dot could be detected in some of the seed lots and silver scurf was found in all lots. Soft rot bacteria (*Pectobacterium sp.*) were found in all samples tested, but in lower levels in the pre-basic seed lots compared to the certified seed lots. *Dickeya solani* was not detected in any of the lots. The levels of bacteria were significantly higher in the samples tested after harvest in comparison to the seed potatoes and samples taken after storage. There was no correlation between black leg observed in the field throughout the season and the amount of bacteria found in the lots after harvest.

Keywords: diagnostics, potato diseases, skin blemish diseases, soft rot bacteria

Session 6
Poster 118

Shift in sensitivity of Danish *Zymoseptoria tritici* populations to triazole fungicides

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Zymoseptoria tritici is the causal agent of Septoria tritici blotch (STB) on wheat and one of the most devastating foliar diseases worldwide. The disease is commonly controlled two to three times during the season with strobilurin, triazole and SDHI fungicides. In recent years, however, a gradual shift towards more strains insensitive to these three fungicide groups has been observed in several countries such as Ireland and the U.K. The decline of fungicide sensitivity has been associated with molecular changes in the different targets of the different fungicide groups. Sensitivity towards triazole fungicides has been seen on an acceptable level in Northern Europe for many years, however, in recent years a gradual decline has also been witnessed here. In 2016, results of the national sensitivity testing showed that EC_{50} values for triazole epoxiconazole were significantly higher than in the years before, indicating a major shift in triazole sensitivity. This development goes hand in hand with the detection of more divers CYP51 *Z. tritic*i variants that might explain this loss in sensitivity. The number of actives available in Denmark for control wheat diseases is low only covering few multisite inhibitors (folpet and mancozeb), few triazole fungicides, few strobilurins as well as a single SDHI (boscalid). Therefore, it remains difficult to combine efficient disease control with anti-resistance strategies. It has however been shown that a well thought through spray plan helps minimising selection for more resistant *Z. tritici* variants.

Keywords: epoxiconazole, Septoria tritici blotch, disease control, CYP51

Session 6
Poster 119

Effect of preparations containing nanoparticles towards chosen fungal pathogens of caraway *Carum carvi* L.

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In recent years interest has increased in the possibility of using preparations containing active substances in the form of nanoparticles to reduce the occurrence of bacteria and fungi pathogenic to humans and different crops. The fertilizers containing nanoparticles of copper, silver, and chitosan: Viflo ® copper, Viflo Chitosol ® Silver ® Viflo Cal S and isolates of *Septoria carvi* Syd. were used to the study. The percent of growth inhibition, development of *S. carvi* colonies and ability of Viflo ® Cal to control plants against infection by *S. carvi* was adopted as the criterion of evaluation of the nanoformulations. *In vitro* it has been shown that the tested formulations with nanoparticles limited the growth and development of the *S. carvi* and Viflo Cal ® S at the concentration of 1 $g \cdot cm^{-3}$ was the inhibitor of mycelial growth. In climatic chamber Viflo Cal ® S protect caraway`s seedlings against the infection by *S. carvi*. *In vivo* Viflo Cal S ® significantly limited the severity of septoriosis and powdery mildew *Erysiphe heraclei* D.C., which contributed to increase the size and quality of the caraway crop. Actually, the study of the effect of nanoformulations on the content and composition of biologically active substances and on the quality of the raw pharmacopeias material of chosen plants were carried out.

Keywords: caraway, fungi, control, nanoformulations

Session 6
Poster 120

Emerging framework for adjusting IPM research to customer value

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Integrated Pest Management (IPM) researchers are challenged to increase the adoption rate of IPM knowledge into practice. Many IPM research leaders believe the solution is in further improvement of innovative IPM tools and knowledge supply. In our opinion, however, the solution is in attention for the motivations of farmers & growers and for the values and incentives of value chain partners. Therefore, in this paper we shift the focus from technology push to market pull.
A flow chart will be presented which shows the dynamics of innovations in food chains. The flow chart starts with social unrest and (5 – 8 years later) results in customer value. The secret of this result is a coordinated action of knowledge partners, farmers & growers and value chain partners. Involvement of civil society organisations, farmers' organisations and retail and food service companies is crucial for bringing this coordinated action to a successful conclusion.
The economic driver in this process is integrating IPM solutions in new product concepts that give access to expensive market segments. New product concepts (e.g. residue-free snack tomatoes in a plastic cup) provide a win-win situation for all partners in the value chain. An analysis of export data revealed 10-20% higher export prices for exports of fresh tomatoes, sweet peppers and apples to the highly demanding German market.
The take-home message for IPM researchers is to focus their work on co-creation of customer value together with input suppliers, farmers & growers and value chain partners. The trendsetters among farmers & growers are already applying the customer value strategy. According to farm accountancy data, these trendsetters manage to realise high gross yields against low crop protection costs. On the other side of the spectrum crop protection costs are high and gross yields are low.

Keywords: adoption rate, dynamics, motivations, incentives, co-creation, value chain

Session 6
Poster 121

Green manure based mulch helps suppress *Phytophthora infestans*

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Nutrient supply and late blight, caused by *Phytophtora infestans* are the major challenges in organic potato production. Copper fungicides are harmful to the environment in high dosages and their ban in Europe is imminent. Some organic farmers reported to us that the use of green manure based fresh mulch would considerably reduce late blight while supplying nutrients to the potatoes. Experiments were conducted in 2014-2016 under organic conditions with natural inoculum using inversion and non-inversion tillage and various green manure mulch sources to verify these effects. No *P. infestans* occurred in 2015 and Data from one experiment in 2014 and two in 2016 are presented.

The experiment in 2014 combined reduced tillage with 10-12 cm fresh winter pea rye based mulch applied right at potato emergence compared to a regular plough based system. In the first experiment in 2016, triticale-vetch and grass clover mulches were compared ton o mulch in a regularly ploughed field. In the second experiment, triticale vetch was grown as pre-crop to potatoes and rototilled to a depth of 5cm prior to potato planting. Half the area was subsequently hilled and covered with a C-rich mulch of fresh triticale, the other half was left unmulched.

In all three experiments, the epidemic onset with *P.* infestans was delayed by a few days and plants died of late blight between 10 to 20 days later when mulched compared to non-mulched plants. Areas under the disease progress curve were reduced by 27 to 38% depending on mulch type and year. Microclimatic conditions in the fields were substantially drier due to the hygroscopic properties of the drying plant materials. However, depending on the weather conditions, mulching increased or reduced potato yields by affecting soil moisture and temperature pointing to a need for system optimization before recommending the technology in practice.

Keywords: late blight, potatoes, mulch, reduced tillage

Session 6
Poster 122

Impact of UV-C radiations of strawberry plants on its sensitivity to *Botrytis cinerea*

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Increasing the resistance of plants by using physical methods remains poorly studied compared to the use of biological or chemical elicitors. The objective of the study is to test the hypothesis that it is possible to reduce the sensitivity of strawberry plants to *Botrytis cinerea* by the application of non-deleterious dose of UV-C radiation on the plant.
Tests were carried out on three cultivars of strawberry. We first showed that these cultivars have different levels of basal sensitivity to *B. cinerea* on leaves. Various treatments combining different doses of UV-C and frequency of application resulted in the selection of non-deleterious doses for strawberry plants. Measures of chlorophyll fluorescence, leaf color and plant growth revealed that doses comprised between 0.4 and 1.70 kJ/m² had not any significant effect on these plant physiological parameters, regardless of the frequency of application and of the cultivar tested.
It also resulted in the identification of hormetic doses that can improve the resistance of leaves against grey mold. UV-C treatments applied on plants at 0.85 and 1.70 kJ/m² 4 times every two days, resulted in a systematic decrease in the sensitivity of the cultivar Candiss to the strain Bc1 of *B. cinerea* (leaf protection between 11 and 27%). But the effect observed is different according to the strawberry cultivar and to the strain of *B. cinerea* used. In future experiments, we envisage to test the effect of these treatments on fruit quality and in their post-harvest sensitivity to *B. cinerea*.

Keywords: strawberry, *Botrytis cinerea*, UV-C radiations, hormetic dose

Session 6
Poster 123

R2E: a participatory experimentation network for a better integration of biocontrol solutions

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The Experimental Excellence Network (R2E) is a collaboration between various research and development organizations, all in close contact with farmers, working together to develop agronomic references in order to develop a multi-efficient agriculture. The main motivations of R2E members are:
- evaluate agronomic innovations in the broad sense (crop protection, nutrition, cultivation techniques, etc., which can open up pathways for agricultural progress,
- to pool technical expertise and experimental means in order to carry out a common R & D program,
- to share references on agronomic innovations, mainly from field experiments,
- ensure homogeneity and excellence of methodologies,
- strengthen the representativeness of acquired references by valuing the diversity of agronomic situations (network dynamics).
For its launch year in 2015, the R2E network has chosen to evaluate the available or in developpment biocontrol solutions for the control Septoria *(Zimoseptoria tritici)* on wheat. In 2016, the thematic field was extended to the control of Fusarium head blight (*Fusarium graminearum*) on soft wheat by biocontrol products. This paper will attempt to present this experience of participatory research, as well as the first results obtained in this framework with sulphur and with *Pythium oligandrum* to control respectively Septoria and Fusarium head blight.

Keywords: participatory experimentation network, wheat, *Zimoseptoria tritici, Fusarium graminearum,* biocontrol, sulphur, *Pythium oligandrum*

Session 6
Poster 124

Exploring the effect of combining crop genetics and fungicidal chemistry to control a major root pathogen of wheat

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Gaeumannomyces tritici is the world's most destructive root pathogen of wheat, causing take-all disease. A newly discovered genetic trait in elite hexaploid wheat, called LowTAB (low take-all build-up), limits take-all inoculum build-up in the rhizosphere. Thus, LowTAB cultivars reduce the risk of take-all disease damaging a consecutive wheat crop, thereby improving crop health and increasing grain yields. This research has investigated the effect of combining LowTAB, with foliar applications of take-all active fungicide, Amistar (active ingredient: azoxystrobin), on take-all inoculum build-up and subsequent disease in second wheats. Nine chemistry regimes were applied to two cultivar treatments in three replicated, fully randomised first wheat field trials in Hertfordshire, UK, between 2013-2015. A post-harvest soil core bioassay measured the resulting amount of take-all inoculum build-up. All three trials were then oversown with a take-all susceptible second wheat cultivar and disease resulting from the first wheat treatments was quantified during grain filling.
Early first wheat sprays of Amistar were found to reduce take-all disease in the subsequent second wheat crops, when measured using the take-all index in a three year combined REML analysis ($P = 0.009$). A mid-season first wheat spray had an immediate reductive effect on take-all inoculum ($P = 0.014$), but no significant effect on second wheat take-all index was observed. We conclude that Amistar, applied in early spray regimes to first wheats, effectively lowered second wheat disease, but when applied as mid-late first wheat sprays, altered the normal take-all disease progression cycle. The LowTAB genetic trait consistently lowered both inoculum ($P<0.001$) and second wheat disease ($P<0.001$), however no synergy with Amistar was identified, indicating the effects of the genetics and chemistry will work additively. The LowTAB trait is currently being genetically mapped in several breeding populations and findings from this research should help towards providing on-farm solutions to take-all disease.

Keywords: *Gaeumannomyces graminis*, take-all, integrated disease management, *Triticum aestivum*, azoxystobin, wheat genetics

Session 6
Poster 125

Towards a more sustainable agriculture: arbuscular mycorrhizal fungi protect wheat against powdery mildew

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The use of arbuscular mycorrhizal fungi (AMF) as resistance inducers and therefore as biocontrol tools could be an innovative alternative to chemicals for controlling plant diseases. Our work aimed at studying the possible protective effect of arbuscular mycorrhization in bread wheat (*Triticum aestivum* L.) against *Blumeria graminis* f. sp. *tritici* (*Bgt*), the obligate biotrophic Ascomycete fungus infecting wheat aerial organs and responsible for the powdery mildew disease. Wheat mycorrhizal inoculation by *Funneliformis mosseae* (*Fm*), under controlled and nutritional optimized conditions, allowed us to obtain concomitantly a mycorrhizal rate of 38%, a significant increase of plant biomass and a protection level against *Bgt* estimated at 78%. These results suggest the induction of systemic wheat defense reactions in response to wheat mycorrhization, *i.e.* Mycorrhiza-Induced Resistance (MIR). This protection is linked to an accumulation of phenolic compounds and hydrogen peroxide at the *Bgt* penetration sites in epidermal leaf cells of mycorrhized plants. Up-regulations of *POX*, *PAL*, *NPR1* and *CHI1* genes encoding for defense markers were also pointed out in leaves of mycorrhizal wheat in the absence of *Bgt* infection. Our study also highlighted the importance of taking into account various environmental parameters to optimize the potential use of AMF as biocontrol agents. The highest protection against powdery mildew was obtained with a 5-fold reduced phosphorus input compared to that recommended in the field, both in a moderately cultivar and in a more resistant cultivar. Moreover, the level of wheat protection depends more on the inoculum type than on the mycorrhizal rate observed in wheat roots.

Keywords: arbuscular mycorrhizal fungi, powdery mildew, wheat, mycorrhiza-induced resistance, biocontrol

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ZnO nanoparticles for postharvest strawberry grey mould control

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It is well known that most of the pathogens can develop high resistance to chemical fungicides. Researchers all over the world are looking for new technologies reducing pesticide consumption. New implements are needed to reduce postharvest losses and control food pathogens. This method are based on measures of zinc oxide (ZnO) nanoparticles. Photosensitization is an innovative approach for eliminating fruit pathogens based on simultaneous use of photoactive compound (photosensitizer) and visible light.

This study aimed to evaluate the efficacy of new measures for reducing contamination of pathogenic fungi in strawberry. The research was carried out at the LAMMC Institute of Horticulture. The photosensitization (PH) evaluated in LED-based light source prototype. Experimental treatments included 1) control, 2) ZnO + PH, and 3) sterile water + PH. Visually healthy strawberry cv. "Darselect" fruits were incubated with photosensitizer ZnO for 1 h and illuminated 30 min. with light (λ=400 nm with an energy density of 20 mW/cm2). Decontaminated fruits assessed after 4 and 8 days of simulated storage at 5-7 ^{o}C.

The experimental results revealed that 4 and 8 days after the ZnO treatment, the number of *Botrytis* infected fruits reduced by 10% and 13%. In water treatment, the incidence of grey mold after a 4-day storage was 9% and 19 % higher compared to the control and ZnO. Data indicated that ZnO nanoparticles reduced strawberry contamination with *Botrytis cinerea*. Such ZnO nanoparticles properties are a promising tool for the development of effective nonchemical fungicides.

Acknowledgement. This work was carried out within the framework of the long-term research program "Harmful organisms in agro and forest ecosystems" implemented by LAMMC.

Keywords: *Botrytis cinerea,* contamination, photosensitizer, Zinc Oxide

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Are sown flower strips an efficient tool to limit viral epidemics and aphid colonization in melon crops?

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The melon aphid *Aphis gossypii* is a serious pest on melon crops causing leaf-curling, stunting and even plant death when colonization is intense. It is also an efficient vector of four viruses frequently observed on melon crops in France: *Cucurbit aphid-borne yellows virus* (CABYV, Polerovirus, Luteoviridae), *Cucumber mosaic virus* (CMV, Cucumovirus, Bromoviridae), *Watermelon mosaic virus* (WMV, Potyvirus, Potyviridae) and *Zucchini yellow mosaic virus* (ZYMV, Potyvirus, Potyviridae). The management of aphids and viruses is all the more challenging that the evolution of the regulation (plan Ecophyto 2018) imposes a progressive reduction of the usage of the phytosanitary products. Innovative strategies are needed to control these bioagressors. The bibliography suggests that the management of field margins could contribute to regulate the populations of aphids and/or their viral load. Indeed, flower strips can participate in aphid biological control by favoring natural enemies and strips of non-host plants can protect crops from non-persistently aphid-transmitted viruses by allowing aphids to probe on healthy plants and thus to lose their virus load before reaching the crops. A pluriannual (2011-2015) and multidisciplinary experiment allowed an evaluation of the potential role of sown flower strips to decrease the risk of aphid colonization and the risk of viral epidemics in melon crops.

Keywords: *Aphis gossypii*, conservation biological control, *Cucumis melo*, integrated pest and disease management

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Bacillus amyloliquefaciens GA1 as potential biocontrol agent of Septoria tritici blotch

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Zymoseptoria tritici is the causal agent of Septoria tritici blotch (STB), one of the most damaging foliar disease of wheat. Conventional fungicides are widely used to reduce STB and related yield losses but chemical pesticides are increasingly controversial. Thus, research efforts are needed to identify alternatives control tools such as bacterial control agents or their metabolites. Among them, strains belonging to the genus *Bacillus* and particularly *B. subtilis* and *B. amyloliquefaciens* are efficient for the biocontrol of multiple fungal diseases. The aim of this work was to determine if the filtrate of a *B. amyloliquefaciens* GA1 72 h culture possesses the ability to protect wheat against *Z. tritici. In vitro* bioassays showed a strong antifungal effect of the filtrate, with a minimal-inhibitory concentration of 6.25 % v/v (in mixture with potato dextrose agar medium used for fungal growth). *In planta* experiments, carried out in the greenhouse on the susceptible cv. Alixan, showed strong disease reductions (up to 98.5 %) on plants treated with the culture filtrate at 100 %, 50 % or 25 % v/v (in distilled water). Cytological investigations revealed a significant impact of the treatments on both spore germination (at day one post-inoculation) and fungal growth (at day five post-inoculation), thus agreeing the *in vitro* findings. Further molecular investigations are in progress to determine the ability of the culture filtrate to elicit plant defense pathways. In conclusion, this study demonstrated a significant potential of *B. amyloliquefaciens* GA1 to be used as a bio-pesticide on wheat against *Z. tritici*.

Keywords: *Zymoseptoria tritici*, *Bacillus amyloliquefaciens*, biocontrol, biopesticides

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Oligosaccharide-based inducer exhibits high protection against *Zymoseptoria tritici* and induces various related-defense pathways in bread and durum wheat

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The aim of this work was to investigate the eliciting and protective effects of preventive treatments with an oligosaccharide-based new formulation (Oligos) against *Zymoseptoria tritici*, a major pathogen of both bread wheat (BW) and durum wheat (DW). Oligos treatment led to strongly reduced fungal hyphal growth on leaf surface, penetration attempts, mesophyll colonization and fructification during *Z. tritici* infection on both tested BW (cv. Premio) and DW (cv. Karim) cultivars. It also drastically decreased CWDE activities by *Z. tritici*, such as endo-β-1,4-xylanase and endo-β-1,3-glucanase during the necrotrophic phase of infection, suggesting their correlation with disease severity and their potential use as markers of resistance inducer efficiency. The expression of defense-related genes such as *GLUC, Chi4, POX* and *LOX,* the activities of GLUC and LOX and the content of total soluble phenolic compounds were enhanced in both non-inoculated and inoculated plants. However, induced PAL activity, H_2O_2 accumulation and polyphenol deposition in mesophyll cells were observed only in inoculated context but not to the same way between both wheats. Overall, Oligos treatment induced the same defense pathways in tested BW and DW cultivars, but not to the same extent, such as GLUC, CAT, LOX and PAL enzymatic activities. Further investigations using additional susceptible and partially-resistant BW and DW cultivars are required to confirm these results. The Oligos resistance inducer presents an interesting activity characterized by high and stable protection efficiency when it is used inappropriate conditions, and therefore could be integrated into alternative control strategies against *Z. tritici*.

Keywords: *Zymoseptoria tritici, Mycosphaerella graminicola*, oligosaccharides, resistance inducer, wheat

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Evaluation of leaf extracts of four plant species against rice blast pathogen (*Magnaporthe oryzae)*

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Rice (*Oryza sativa*) is one of the most popular food crops in Nigeria. Its successful production has been drastically affected by blast disease caused by *Magnaporthe oryzae*. *In vitro* control of the pathogen by four medicinal plants *(Carica papaya*, *Azadirachta indica, Calotropis procera* and *Anacardium occidentale)* was assessed in this study. The extracts of the plants were prepared using aqueous and methanol, and agar well diffusion method was used to assess the toxicity of each extract. The pathogen was isolated from rice infected with blast disease. The results revealed the presence of one or more phytochemicals in each of the plant extracts. Among these were alkaloids, tannins, flavonoids, saponin, anthocyanin and phenol. All the extracts inhibited mycelia growth of *M. oryzae*. The potency of all the extracts increased with increasing concentration in the order; 50mg/ml<100mg/ml/150mg/ml. The inhibitions by methanol extracts were higher and significantly different ($P>0.05$) from aqueous extracts. At the highest concentration tested (150mg/ml), *A. occidentale* and *C. procera* gave the highest inhibitions (99.0mm and 98.6mm respectively) which were not significantly different ($P<0.05$) but different from *C. papaya* and *A. indica* (89.1mm and 90.4mm respectively). However, in all, *A. occidentale* aqueous and methanol extracts gave the highest percentage growth inhibition of the pathogen at all levels of concentrations tested while *C. papaya* aqueous and methanol extracts though effective were the least. Therefore, field trials of these four medicinal plants on the control of rice blast disease are recommended since they are easy to obtain and the extracts could easily be made via a simple process of maceration or infusion, they could be cheaper substitute for conventional drugs in controlling rice blast disease.

Keywords: blast, potency, extract, phytochemicals, pathogen

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Salt and thermos-tolerant *Trichoderma asperellum* isolate from Oman is a potential antagonist of root rot pathogens

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One of the biggest threats to maize in Oman are root rot diseases. *Trichoderma* species are known to be natural antagonists of many pathogens and are widely used in biological control of fungal plant diseases. There are commercial bio control fungi but they might not perform optimally under high salinity and high temperature conditions. Two *Trichoderma asperellum* isolates from Omani organic compost and a maize rhizosphere were evaluated for antagonistic effects against four known plant pathogens causing maize root rot in Oman: the fungi *Fusarium fujikuroi*, *Botryodiplodia theobromae,* and *Rhizoctonia solani* and the oomycete *Pythium arrhenomanes*. A commercially available biocontrol isolate of *Trichoderma harzianum* was used as control.
The local isolates showed antagonistic effects and parasitism against the three fungi similar to the commercial species. In contrast, biocontrol activity against *P. arrhenomanes* was significantly higher than for *T. harzianum*. Scanning electron microscopy showed that the *T. asperellum* isolates coiled around the pathogens and led to hyphal collapse at the interaction zone suggesting direct hyperparasitic interactions.
Effects of different salinity concentrations (24µS/cm-50 dS/m) and temperatures (5-40°C) on the local *T. asperellum* and the commercial *T. harzianum in vitro* were assessed. All isolates were negatively affected by increasing salinity. However, the growth rates of the local isolates were significantly higher than that of the commercial *T. harzianum* at all salinity concentrations. Growth of the local isolates was comparable to the commercial agent at temperatures up to 30°C but it was significantly higher at 35°C.
These results demonstrate that the local *Trichoderma* local isolates are more thermo- and salt-tolerant than the commercially available product and, therefore, could be potentially effective bio control agents for local use.

Keywords: bio-control, *Trichoderma asperellum, Maize* Root rot, antagonism

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***Aspergillus westerdijkiae* with strong activity against *Pythiaceae* causing dieback of apple trees**

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Dieback disease of apple trees caused by *Pythiaceae* (*Pythium* sp., *Phytophthora* sp.; oomycetes) is an important soil-borne disease reducing apple (*Malus communis*) production in Tunisia. It causes severe damages and tree losses in numerous apple orchards. This disease causes cankers, necrosis and rot in the collars and roots.

The present study aimed at investigating the biocontrol potential of antagonistic fungal (e.g., *Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp.) or bacterial isolates (*Bacillus* spp.) against *Pythium* and *Phytophthora* sp, to determine their phylogenic position, and to characterize the antifungal compounds produced.

The antagonistic effects of selected biocontrol agents (BCAs) were evaluated by different screening methods *in vitro*. Using the dual culture technique on solid medium as well as using culture filtrates, we concentrated our study on those isolates for which we were able to detect secreted antifungal activity inhibiting *in vitro* growth of tested *Pythiaceae* by over 50%. The results of this study showed *in vitro* efficacy of two isolates of *Trichoderma* spp., one isolate of *Aspergillus* spp. and two strains of *Bacillus* spp against *Pythium* and *Phytophthora* spp.

The highest activity was found for an *Aspergillus westerdijkiae* isolate whose culture filtrate inhibited the mycelial growth of *Pythium undulatum*, *Phytophthora unidata* up to 100 %. In order to identify the active molecule(s) we purified and characterized the culture filtrate by different chromatographic and spectroscopic techniques including HPLC and LC/MS.

We selected a unique fraction from culture filtrates with high inhibitory activity. Its chemical characterization is ongoing. These encouraging results incite us to proceed through *in vivo* assays of the selected *Aspergillus westerdijkiae* isolate against *Pythium* sp. on apple plants.

Keywords: *Malus communis*, *Pythiaceae,* BCAs, antifungal activity, HPLC, LC/MS

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Antifungal potential of hops extracts against the wheat pathogen *Zymoseptoria tritici*

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Research on alternative methods to conventional pesticides against crop pathogens is an important challenge. Here, we tested the potential of extracts and compounds from hops to be used as biofungicides towards *Zymoseptoria tritici*, the main pathogen on wheat crops today in Europe. Hops is a food and medicinal plant, rich in prenylated phenolic compounds which are responsible for numerous biological activities including antiviral, antibacterial and antifungal properties. Hops has not yet been studied for the biocontrol of plant pathogens. We first assessed the antifungal potential of crude hydro-alcoholic extracts of different parts of hops plant (leaves, stems, roots and female cones), as well as the essential oil of female cones. The antifungal assays were performed using a spotting test carried out in Petri dishes containing potato dextrose agar medium amended with different concentrations (1.25, 0.62, 0.31, 0.15 and 0.07 $g.L^{-1}$) of each extract. Dose-response curve analyzes revealed that only the hydro-alcoholic extract of female cones, as well as their essential oil, significantly decreased fungal growth, with half-maximal inhibitory concentrations (IC_{50}) of 0.73 $g.L^{-1}$ and 0.36 $g.L^{-1}$, respectively. Furthermore, a fractionation of the hydro-alcoholic crude extract from female cones using centrifugal partition chromatography allowed the purification of phenolic compounds. Antifungal assays using the purified products revealed that the prenylated chalcone desmethylxanthohumol, as well as the acylphloroglucinol derivative *co*-humulone, are responsible for part of the antifungal effect of female cones. The essential oil is being characterized in order to highlight the antifungal terpenic compounds involved in the activity against *Z. tritici*.

Keywords: biofungicides, hops, wheat, *Zymoseptoria tritici*

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Multiple screening approach for the selection of efficient biological control agents against *Botrytis cinerea* on tomato

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One hundred and twenty one bacterial isolates collected from tomato stems and from soil in the Bejaia region (Algeria) were screened for their potential to control gray mold on tomato. The bacteria were initially tested for their direct *in vitro* effect against two strains of *B. cinerea*. Based on these results, 37 isolates showing *in vitro* inhibition of *B. cinerea* mycelial growth (ranging from 0% to 69% inhibition) were chosen for further analysis. These isolates were identified based on *16S rDNA* sequencing. Those identified as potentially pathogens for humans, mammals or plants and able to grow at 37°C were discarded. Twenty five were then selected to evaluate their effectiveness as biological control agents against *B. cinerea* using a tomato plant bioassay. Comparisons of *in vitro* and *in planta* screening methods highlighted the absence of correlation between these methods and confirm that a single screening strategy is not sufficient to select efficient biological control agents.
Among the 25 isolates, 8 exert high and significant antagonistic activity against two strains of *B. cinerea*. Among them, three bacteria belonging to the *Pseudomonas* genus were finally selected for their significant and stable efficiency at 3 concentrations (10^7, 10^8 and 10^9CFU/mL) to reduce the lesion development of *B. cinerea* on tomato stem (% protection comprised between 60 and 100%), their ability to grow at 15°C and their inability to grow at 37°C. These three isolates are also able to reduce significantly the sporulation of *B. cinerea* on tomato stem.
This study is a first step in the selection of biological control agents able to protect the tomato against *B. cinerea* and adapted to the greenhouse conditions in Algeria.

Keywords: *Botrytis cinerea*, tomato, biological control agents, screening, *Pseudomonas* spp.

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In vitro effect of essential oils on the *Colletotrichum gloeosporioides* development

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Colletotrichum gloeosporioides, causal agent of sweet pepper anthracnose, is one of the most important pathogens of this crop. The present study was carried out in order to identify an alternative option for this fungus control, evaluating the effect of essential oils (EO) on the fungus *in vitro* development. The experiment, performed in duplicate, was carried out in a completely randomized design in a factorial scheme 8 x 3 [8 essential oils (rosemary, citronella, clove, copaiba, eucalyptus, peppermint, basil and tea tree) x 3 concentrations (0.25 %; 0.5 % and 0.75 %) and one control treatment (potato-dextrose-agar medium - PDA), with 3 replications. The oils were added to PDA medium at predetermined concentrations. A disc of 0.5 cm diameter of PDA medium with the fungus was placed in the center of each petri dish containing the treatments. The plates were maintained at 22 °C and 12 hour-photoperiod. To evaluate the inhibitory effect of the oils, mycelial growth and sporulation for each treatment were determined. In both experiments, clove, citronella, eucalyptus, mint and basil essential oils inhibited the fungus mycelial growth and sporulation completely in all concentrations. Tea tree EO showed the same inhibitory effect in concentrations higher than 0.50%, while rosemary EO provided total inhibition at 0.75%; for both EO there was high sporulation. Copaiba EO had non-satisfactory results. According to the results, it can be concluded that clove, citronella, eucalyptus, mint and basil essential oils have a potential use in the alternative control of *C. gloeosporioides*.

Keywords: Anthracnose, natural fungicide, sustainable development

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Antagonism of *Trichoderma.* sp with *Pestalotiopsis*. sp in Tunisia

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The fungi are one of the main causes of the diseases of trees. The symptoms of dieback include a fall and a yellowing of foliage, a drying and necrosis at the level of branches, cankers, deformations, of a blackish fluid and flow of rots at the level of the trunks (Ben Jamâa, et.Al., 2005; Hasnaoui and al., 2008; Barky & Abourouh, 1996; Barky et al. 1999; Franceschini et al., 1999, 2002). The forest of Henchir Kort, (northeast of the Tunisia) has suffered heavy infestation since 2012. Symptoms of wilting were noted on the pine (*Pinus pinea*) and several other species of scrub oak scale (*Quercus coccifera*). In October 2016, attacked samples with symptoms of necrosis and dryness have been collected shrubs of oak scale. The pathogenic fungus *Pestalotiopsis*. sp has been isolated from these lesions. The Koch's postulate has been verified. The antagonistic action was assessed in vitro. The results show that in mixed culture with *Pestalotiopsis. sp*. isolate of *Trichoderma.* sp has shown an effect from the inhibition of Mycelial growth (fungistatic effect) to the degradation and the disappearance of the mycelium of *Pestalotiopsis* (mycoparasitism and fungicidal effect). The study continues with a benchmark test of the effectiveness of the natural antagonist

Objective: The present work is the search for a biological control method using a natural antagonist.

Materials and methods

1. Isolation and identification of fungi (Isolation were made according to the technique conducted by Franceschini et al. 2005).
2. Test of pathogenic pathogenesis test was conducted using the method of inoculation of Linaldeddu et al. (2014).
3. Test of confrontation (Benhanou and Chet, 1996).
4. Assessment of the survival rate of *Pestalotiopsis*. sp

Results and conclusion

1. The koch's postulate has been verified: all of the inoculated twigs were infected with *Pestalotiopsis*.sp.
2. The test of direct confrontation in vitro between *Pestalotiopsis*. sp and *Trichoderma* sp. has show a fongistatic effect and fungicidal effect.
3. The assessment of the percentage of survival of pathogenic fungus test reveals that *Pestalotiopsis.* sp. has a rate of 0 %.

Keywords: kermes oak, necrosis, *Pestalotiopsis*, *Trichoderma*, antagonistic

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Fitness competitiveness and sensitivity to fungicides of *Monilinia laxa* and *Monilinia fructicola* isolates from Serbia

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Brown rot is one of the most severe pre- and postharvest diseases with a worldwide distribution. Since *M. fructicola*, the most destructive pathogen of the genus *Monilinia*, has recently been introduced into Serbia and many other European countries, many studies were conducted to evaluate differences in characteristics of *Monilinia* species that could have an impact on establishment and survival of the pathogen in new areas. The aim of this study was to assess the capacity of *M. fructicola* to repress and replace *M. laxa* in Serbia, based on the comparison of the isolates in terms of fungicide sensitivity (iprodione, tebucanozole, prochloraz, chlorothaloni, azoxystrobin, fluopyram, and boscalid), growth rate and virulence at different temperatures. The results showed that the isolates of *M. fructicola* were significantly less sensitive than *M. laxa* to the following fungicides: iprodione, tebucanozole, chlorothalonil, azoxystrobin, fluopyram, and boscalid. Moreover, *M. fructicola* isolates displayed a wide range of sensitivity, whereas *M. laxa* isolates exhibited little variation in sensitivity to all the tested fungicides. In addition, growth rate and virulence of both species were significantly affected by the temperature. The lowest tested temperature (5°C) was favorable for *M. laxa* growth rate and virulence, whereas at 30°C *M. fructicola* grew faster and had higher lesion expansion rate. These results provide an additional explanation for significant changes in the population structure of *Monilinia* spp. in Serbia after the introduction of *M. fructicola*, esspecially in peaches and nectarines that are ready for harvest during hot summer time.
Project III46008.

Keywords: fungicide sensitivity, competitiveness, species displacement, stone fruit

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Control of powdery mildew (*Blumeria graminis*) in cereals using Serenade® ASO

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Powdery mildew (*Blumeria graminis*) is a serious pathogen of cereal crops in many parts of the world. Traditionally this disease has been controlled by using resistant cultivars or by applying fungicides. In recent years more focus has been put on biological control agents (BCA's) as alternatives to chemical fungicides for control of diseases in various crops, including cereals. The biofungicide Serenade® (*Bacillus subtilis* strain QST 713 from Bayer Crop Science) was investigated for its potential for control of powdery mildew in cereal crops. *Bacillus subtilis* is a rhizobacterium that can form endospores, produce several different antibiotics and produce microbial disrupters of pathogen cell membranes. The product is approved for use in the European Union, and the main targets so far have been *Botrytis cinerea* and *Erysiphe* species in vegetables and strawberries.
Field trials carried out in wheat and barley crops in Denmark have shown moderate to good control of powdery mildew using Serenade® when repeated treatments have been applied. Data from greenhouse trials have shown that correct timing is very important for good control. Best control is obtained if treatments are applied just around the time of infection. Only minor or no dose responses were seen when using 2, 4 or 6 l Serenade® per ha. Both in the field and the greenhouse trials the traditional fungicides (triazoles) provided superior control compared to treatments with Serenade®. The challenge for BCA's in cereal crops is to find means of integrating their use with traditional chemistry and understand their role in a combined disease management scenario. The results so far indicate that the BCA's can't be stand-alone solutions for conventional farmers, but their use needs to be exploited further also as a means of reducing the risk of development of fungicide resistance. For organic farmers the main challenge will be to know when the crop is at risk of attack as a delayed spraying has been seen to provide less effective control.

Keywords: biological control agents, efficacy, wheat, barley

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Poster 139

Induction of potato defense responses and reduction of symptoms from *Phytophthora infestans* by two different elicitors

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Phytophthora infestans is an oomycete responsible for potato late blight. Due to aggressiveness of this disease, the only way to control is with many fungicide applications. Thus, a major scientific challenge is to develop alternative methods as plant defense induction to reduce pesticide treatments. In this aim, our study focus on the potential efficiency of defense elicitors to protect potato against *P. infestans*. Then a concentrated culture filtrated (CCF) from *P. infestans* (endogen elicitor) and a green algae's (*Ulva* spp.) extract (exogenous elicitor) were tested on two potato genotypes: BF15, moderate susceptible to *P. infestans* and Désirée, moderate resistant. Potato plants have been treated with the elicitors at different concentrations and 48h later detached-leaves were inoculated with *P. infestans* at 5.10^4 sporangia.mL^{-1}. Induction of defense responses has been evaluated after elicitation on non-inoculated leaves by a transcript analysis of twelve potato defense genes. The quantity of symptoms has been evaluated until seven days after inoculation on treated and untreated leaves by the measure of necrotic areas. Our results showed that CCF induced more strongly defenses genes in BF15 than in Désirée genotype but none reduction of symptoms was measured after CCF application. However, algal extract induced the expression of genes differentially in both genotypes. But a significant reduction of necrotic area was only observed on BF15 genotype. This reduction could be explained by the strong induction of Phenylalanine Ammonia-Lyase gene and to a lesser degree by the induction of Tyramine N-hydroxycinnamoyl Transferase gene. Indeed, these two genes are implicated in the phenylpropanoid and salicylate pathways, which produce antimicrobial components. To verify this hypothesis, metabolic and proteomic analysis are being tested an attempt to correlate defense induction and effective protection in different interactions between plants and pathogens.

Keywords: biocontrol, plant immunity, Potato late blight, *Solanum tuberosum*

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Effect of essential oils on the apple scab causative organism *Venturia inaequalis*

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Causal agent of Apple scab *Venturia inaequalis* it is the one of the most important pathogen limiting apple fruit production. Decreasing number of fungicides and active substances, as well as the occurrence of resistant pathogen populations and increasing demand for biological fruit production, leading us to develop new fungicides on a natural basis. In this work the efficiency of 13 essential oils from *Mentha spicata, Cymbopogon citratus, C. winterianus, Litsea cubeba, Thymus vulgaris, Pelargonium graveolens, Rosmarinus officinalis, Eugenia caryophyllus, Ocimum basilicum, Cinnamomum ceylanicum, Lavandula hybrida, Origanum majorana* and *Cin. camphora* on the several isolates of *Venturia inaequalis* have been tested. Best results are reached essential oils from plants of *T. vulgaris* and *E. caryophyllus*, which significantly reduced the growth of the organism and thus give a basis for further work in vivo.
This study was supported by the Czech Ministry of Agriculture, project numbers QJ1510353.

Keywords: Apple scab, *Venturia inaequalis*, essential oils, biofungicides, *Thymus vulgaris*, *Eugenia caryophyllus*

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The protection of cereals by fungicidal essential oils extracted from higher plants

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Botanical pesticides are substances derived from plants with a wide range of utilization. We tested effect of essential oils extracted from aromatic plants on inhibition of mycelium growth of phytopathogenic fungi attacking cereals. In our study (i) the laboratory tests with phytopathogenic fungi *in vitro*, (ii) growth chamber biotests with plants and (iii) the field small plot experiments using of artificial inoculation were conducted.
In the laboratory tests the inhibitory effect of essential oils on the mycelial radial growth of fungi was tested by the agar dilution method. The effect of essential oils extracted from *Pimpinella anisum*, *Thymus vulgaris*, *Pelargonium odoratissimum*, *Rosmarinus officinalis* and *Foeniculum vulgare* were tested on the fungi *Oculimacula yallundae, Microdochium nivale, Zymoseptoria tritici, Pyrenophora teres* and *Fusarium culmorum.* The best antifungal activity was demonstrated by *Thymus vulgaris*. The most prevalent compounds of *T. vulgaris* were thymol (44.60 %), p-cymene (21.94 %) and γ-terpinene (7.80 %).
The biotest with artificially inoculated (by *F. culmorum*) wheat seed were conducted in growth chamber. Test plants were cultivated in hydroponic media amended by essential oils, the same as in laboratory test above (0.1% emulsion). The best antifungal activity was demonstrated again by essential oil extracted from *Thymus vulgaris*.
Efficacy of spray microencapsulated formulation of the essential oil from *T. vulgaris* to the ears was tested in the field experiment. Wheat plants were artificially inoculated by *F. culmorum*. Mycotoxin content in harvested grain was analysed using ELISA. Level of deoxynivalenol was significantly reduced after treatment with oil from *T. vulgaris. W*ork was supported by Ministry of Agriculture of the Czech Republic projects QJ1310226, QJ1310091 and RO0211.
Our results suggest that the essential oil from *T. vulgaris* have great potential for safe and environmentally friendly treatment cereals against to fungal pathogens.

Keywords: botanical pesticides, *Thymus vulgaris*, phytopathogenic fungi, wheat, barley

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Efficacy of fungicides and biofungicides in the control of Verticillium Wilt on pepper

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Verticillium dahliae is considered to be one of the most destructive soilborne plant pathogens. Management of the pathogen is difficult, because of its endophytic growth and long persistence in soil. The objective of this study was to examine the possibilities of Verticillium wilt control in pepper by using conventional fungicides (thiophanate-methyl, difenoconazole, fluopyram, azoxystrobine, prochloraz) and two commercially available biopesticides based on *Bacillus subtilis* strain and tea-tree oil. *In vitro* sensitivity of *V. dahlia*e isolate, derived from infected pepper plant and indentified based on pathogenic and morphological features, to the tested fungicides and tea-tree oil was determined in radial growth experiment on PDA medium supplemented with a range of fungicide concentrations. The concentration that inhibited mycelial growth by 50% compared to the control (EC_{50}) was calculated. The efficacy of the tested products *in vivo* was evaluated on inoculated pepper plants under greenhouse conditions. The results of the *in vitro* experiment showed that difenoconazole and thiophanate-methyl were the most toxic fungicides to the isolate (EC_{50}=0.02 mg/l and 0.03 mg/l, respectively), while, under greenhouse conditions, the highest efficacy was recorded for thiophanate-methyl (83.1%). The essential oil and *B. subtilis* – based product exhibited low efficacy in controlling the disease (46.2% and 35.4%, respectively). The obtained results revealed that neither fungicide nor biofungicide treatments could provide adequate supression of the disease without combination with other control strategies. Project TR31043.

Keywords: *Verticillium dahliae*, pesticide control, *Bacillus subtilis,* tea-tree oil

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SpotIT – IT-solutions for user friendly IPM-tools in management of leaf spot diseases in barley and wheat

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Leaf diseases are a major threat to cereals in the Nordic-Baltic area and fungicides are widely used for reducing yield losses. The project SpotIT is a Nordic-Baltic initiative started in 2017, to provide cereal farmers with better models for predicting leaf spot diseases in wheat and barley, aiming for user-friendly dissemination of model outputs through locally adapted IPM-tools. The Norwegian Open Source technology platform VIPS will provide a trans-national facility for model testing and validation, using input data from all participating countries. VIPS will also provide a basis for efficient, user friendly and low-cost expansion of locally adapted decision support systems (DSSs) with a trans-national sharing of knowledge and methods.

Despite development of national DSSs, farmers do not always use the novel tools. Data from previous studies in Denmark, Norway and Finland suggest that relatively few farmers use DSS directly, while agricultural advisors are the main target group. The main approach of SpotIT is to identify user needs and expectations, understand farmers' decision-making and suggest solutions for dissemination of plant protection risks in each country or region.

End-user preferences will be investigated to identify factors to promote increased use of local DSS and implementation of IPM tools. The choice of risk models for leaf diseases in wheat and barley will be based on the user needs as well as the performance of the models under local conditions. We are currently compiling a database of model candidates, which will be presented during the conference. The aim is to test them based on historical data in the different regions. Field tests will be made in cooperation with farmers and extension service to validate the models in different countries. While this project will focus on leaf spot pathogens, the resulting DSS platform can easily be used for other host, pest or pathogen systems.

Keywords: integrated pest management, DSS, VIPS, cereals

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Integrated crop solutions to offer differentiated solutions

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Many diseases which include fungi, oomycetes and bacteria significantly impact crop production and quality. In addition growers are continually facing new emerging or re-emerging diseases, such as soybean rust in Brazil (*Phakopsora pachyrhizi*), citrus greening in California (*Candidatus Liberibacter*), Esca in European vineyards (complex of fungi) or rye ergot (*Claviceps purpurea*) in France. In top of that the industry is facing increasingly challenging regulatory and societal demands in a changing world (*eg* increasing population, changing consumption patterns, climate change, unsufficient storage) making food safety at risk. In this complex environment, innovation in a modern agriculture is key to support these new challenges and integrated crop solutions will be necessary to reach the worldwide demand.
At Bayer, to help farmers secure their harvest and enhance the public perception of their job, we focus our research and development towards a sustainable approach using all tools of a modern agriculture: small molecules, biologicals, biotechnologies including native or GM traits, SMART breeding, precision agriculture and services, systematically accompanied by full stewardship support. Diverse examples of these approaches will be presented and discussed.

Keywords: diseases, integrated crop solutions, farmers, food quality

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Poster 145

Use of compost to partially substitute non renewable growing media and suppress soil-borne pathogens on potted vegetable plants

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Composts are expected to suppress plant diseases, according to the type of wastes, the composting process, the chemical and microbiological composition. Suppressive composts are generally applied as soil improvers, while it is necessary to develop specific compost based growing media for applications on potted plants.
The aim of this research was to evaluate the suppressiveness of compost/peat growing media compared to peat.
A commercial growing media, "Hortofan", made of 20% v/v compost, peat and pumice was tested. Suppressiveness was tested in greenhouse on potted plants against *Pythium ultimum* on cucumber, *Phytophthora nicotianae* and *Fusarium oxysporum* f. sp. *lycopersici* on tomato. Pathogens were mixed into the substrate at 1 g of biomass on wheat kernels L^{-1} 7 days before seeding or with chlamydospores talc at $1x10^4$ UFC/g of substrate. Seeds of cucumber and tomato were sown into 2 L pots in greenhouse and five pots were used for each treatment. A commercial peat based growing media was used as control. The number of alive plants and weight of above ground biomass were measured 20-30 days after seeding.
Cucumber and tomato biomass significantly increased up to 40-50% with "Hortofan" compared to control. The number of diseased tomato plants in substrates inoculated with *P. nicotianae* was significantly reduced by 40% and the number of diseased cucumber plants by 30% compared to the peat substrate. Fusarium wilt of tomato was reduced by 60% in plants grown with "Hortofan".
The growing medium "Hortofan", thanks to its composition based on high quality compost, improved plant development and controls *Pythium ultimum* on cucumber, *Phytophthora nicotianae* and *Fusarium oxysporum* f. sp. *lycopersici* on tomato. Compost is a valuable component of growing media that can partially substitute non renewable sources and suppress soil-borne pathogens.

Keywords: tomato, cucumber, peat, *Pythium*, *Fusarium*, *Phytophthora*

Session 6
Poster 146

Attempts to use *Coriandrum sativum* essential oil to reduce seed pathogens

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The increasing resistance to chemical fungicides drawn attention to the natural products antimicrobial activity. Essential oils are biodegradable and eco-friendly botanical products that are accumulated in various plants. The aim of this study was to evaluate antimicrobial effect of coriander essential oil against horticultural crops seed-borne pathogens. The research was carried out at the LAMMC Institute of Horticulture. The *Coriandrum sativum* essential oil was extracted from local material. The *C. sativum* essential oil efficacy against seed-born fungi was evaluated by planting naturally contaminated onion and cucumber seeds on Petri plates containing potato dextrose agar (PDA). The PDA was cooled to 45 ^{o}C, and essential oil was added: 200 µl/l, 400 µl/l, 600 µl/l, 800 µl/l and 1000 µl/l. The antifungal activity of *C. sativum* essential oil on cucumber and onion revealed that several concentration differently inhibited bacterial and fungal pathogens. Evaluation of the efficiency of essential oil on seed-borne pathogens at concentration of 1000 µl/l completely inhibited the pathogens. The concentration of 200 µl/l least inhibited bacterial and fungal pathogens. The essential oils as bio-fungicides would help to control seed-borne pathogens without increasing chemical fungicides resistance.
Acknowledgement. This work was carried out within the framework of the long-term research programs “Horticulture: agro-biological basics and technologies” and “Harmful organisms in agro and forest ecosystems’ implemented by LAMMC.

Keywords: antifungal, coriander oil, inhibition, pathogens

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Investigations to control *Oculimacula yallundae*, the eyespot causing pathogen, using variety resistance and fungicide application

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As a result of the expansion of the cereal cultivation area, in particular of winter wheat, an intensified attack with stem diseases is increasingly being identified. In paticular *Oculimacula yallundae* and *Oculimacula acuformis*, damages the hosts and inhibit the nutrient uptake, which leads to lodging and yield losses. This danger of contamination is strongly influenced and promoted by the changing weather over winter.

For this study, approaches to control have been tested by the cultivation of resistant varieties and by the use of effective fungicides. In this context, it was also necessary to investigate the sensitivity of the pathogen and its alteration against the active substances.

In laboratory tests, the active substances boscalid, fluxapyroxad, cyprodinil, prothioconazole, prochloraz, metrafenone and pyriofenone were tested in vitro against concentrations of 0.01, 0.1 1.0 and 10.0 ppm against *Oculimacula yallundae*. After 31 days, the highest efficiencies of about 80% were achieved by boscalid and fluxapyroxad.

The efficacy of the active substances boscalid, fluxapyroxad, cyprodinil, prothioconazole, prochloraz, metrafenone and pyriofenone in the wheat cultivars Atomic, Partner, Ritmo and Tobak were investigated in ad planta experiments with artificial infection. The species Atomic and Partner were equipped with the resistance gene Pch1.

In young plant tests, the active ingredients Boscalid> Fluxapyroxad> Cyprodinil showed the highest efficacy with approximately 55%. Only individual symptoms could be determined for the Atomic and Partner, whereas a significant root rot was assessed in the highly susceptible cv. Tobak.

The investigations show that in practice the cultivation of resistant varieties is the basis for the prevention of primary attack and the secondary spread in the stalk. By combining with effective fungicides the efficacy in the control of Oculimacula yallundae can be further increased.

Keywords: *Oculimacula yallundae*, variety resistance, fungicide control, inoculation test

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Effects of organic fertilizer and leaf nitrogen content on Leaf spot disease development in grape cv. 'Campbell Early' caused by *Pseudocercospora vitis*

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The table grape cultivar "Campbell Early", widely cultivated in most South Korea region and some Japan region, has a unique flavor and taste compared to European table grape cultivars and accounts for more than 70% of table grape market in South Korea. "Campbell Early" is very sensitive to soil nutrients and needs efficient soil nutrient management from early growth stage to harvesting season, so most of cultivating techniques are focused on winter and summer pruning and selection of fruiting number before harvesting for efficient nutrient uptake and most of disease control measures were depend on bordeaux mixture.
One of characteristics of "Campbell Early" is that this cultivar is highly resistant to powdery mildew and white mildew, while most of European cultivars are susceptible to them. However, one of major weaknesses in "Campbell Early" is its susceptibility to grape leaf spot disease caused by *Pseudocercospora vitis*. Grape leaf spot disease is a major issue especially in organic grape cultivation practice, due to limited supplement of organic fertilizer during growing season and shortage of organic-allowed material for disease control before harvesting. To examine the relationship between soil nutrient supply and disease development, some kinds of organic fertilzers applied during "Campbell Early" cultivation and chemical composition such as nitrogen and carbon contents in leaf during mid-growth season were analyzed in this study.
Leaf nitrogen contents in early July season had less effects on disease spot number but correlated negatively with disease spot area, while the ratio of carbon to nitrogen showed positive correation with it.
In Fish meal organic fertilizer applying plot, the number of appeared spot and area was higher than Expeller cake fertilizer and Blood meal fertilzer plot before growing season and even worsened the degree of disease in without organic fertilizer plot. When applying Expeller cake or Blood meal fertilizer, leaf nitrogen contents were 2.26~2.48% and spot area was 76%, but without organic fertilizer, leaf nitrogen contents were 1.91~1.94% and spot area increased to 94%.
In conclusion, we suggest that for proper control of grape leaf spot disease in cv. "Campbell Early", it is necessary to take appropriate measures not only on the use of control agent but also on vine nutrients.

Keywords: Campbell Early, *Pseudocercospora vitis*, leaf spot, orgnic fertilizer, nitrogen

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Biofungicide activity of *Juncus maritimus* extracts and effusol against *Zymoseptoria tritici*, the causal agent of Septoria tritici blotch of wheat

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Zymoseptoria tritici is today the most frequently occurring pathogen on wheat crops in Europe. The wide use of synthetic fungicides caused an emergence of fungal resistant strains and potentially negative impacts on the environment. Searching for eco-friendly biofungicides is an important challenge and a promising alternative strategy to such products. In this context, we assessed the biocontrol potential of extracts from eight extremophile plant species from Tunisia against *Z. tritici*. Crude methanolic extracts from different parts of these plants were prepared and assessed for their antifungal activity using a spotting test performed in Petri dishes containing potato dextrose agar medium amended with different concentrations of each extract. Extract from a halophyte plant from the rush family, *Juncus maritimus,* showed the highest activity when collected on a specific substrate. In extremophile plants, the production of secondary metabolites is often influenced by abiotic conditions. Thus, we collected *J. maritimus* rhizomes at different vegetative stages, at different periods and from different substrates to compare their antifungal activities. HPCL-UV analyses of extracts showed differences in chromatogram profiles, suggesting that the plant environment, especially the substrate of the soil, should be taken into account to identify great sources of natural antifungal products. From the most active extract, a 9,10-dehydrophenanthrene derivative, effusol, absent from other *J. maritimus* samples, was purified. This product showed a strong antifungal activity against the pathogen, with a MIC of 19 $mg.L^{-1}$ and an IC_{50} of 9.98 $mg.L^{-1}$. Therefore, this phenanthrene derivative could be a promising biocontrol molecule against *Z. tritici*. Further investigations would allow a deeper understanding of the modes of action of effusol against the pathogen.

Keywords: biofungicides, extremophile plants, *Juncus maritimus,* wheat, *Zymoseptoria tritici,* effusol

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Seed coating with natural biostimulants: a practical tool for control of wheat diseases

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Septoria leaf blotch and Fusarium head blight are two major diseases causing severe losses in durum wheat. Coating seeds with beneficial micro-organisms and plant extracts appears to be a promising approach to maintain the productivity of plants under stress condition. In this study, we evaluated the endophytic bacterium, *Burkholderia phytofirmans strain PsJN*, thyme essential oil, the antagonist *Trichoderma harzianum*, the yeast *Meyerozyma guilliermondii*, and their different associations for their ability to control the diseases mentioned both under controlled conditions and in field. Seeds of a sensitive Tunisian cultivar of durum wheat "Karim" were coated with *B. phytofirmans* (10^8 CFU/ml) and *T. harzianum* (10^6 spores/ml), thyme essential oil (5 ppm), *M. guilliermondii* (10^8 spores/ml). Under controlled conditions, Septoria leaf blotch was monitored in pots while Fusarium head blight was assessed in hydroponic system. Treatments reduced pycnidial coverage of septoria to 10% compared to control (40%). Cytology and enzymatic analysis showed that these treatments enhance plant resistance with increased catalases activity, reduced peroxidases activity and H_2O_2 levels and reduced fungal colonization and development in leaf cells. In field, coated seedlings showed a reduced septoria leaf blotch attack to an average 10-20% compared to control (43%). As for Fusarium head blight, under controlled conditions, severity was reduced of about 30%, with reduced peroxidases activity in roots, enhanced phenolic compounds content in leaves and roots and reduced colonization and macroconidia abundance in root cells.

Keywords: Septoria, *Fusarium*, *Burkholderia, Trichoderma,* thyme, *Meyerozyma*

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Biological activity of selected essential oils against *Alternaria dauci*

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Essential oils in plant protection have got already thousands years old history, but their mode of action and all the possibilities of their use are the main object of the researchers in last decades. These days is a need to find alternative method to synthetic pesticides because final customers call for ecological commodities and also for methods of harvesting, which are friendly to nature. Pesticides on the base of essential oils can be ecological form of synthetic pesticides which can get at qualities of conventional production.
Objects of the research are *Alternaria dauci* on carrot. On the pathogen were tested 16 essential oils (EO) from different plants. The experiments were carried at laboratory conditions, but also in field conditions. The biggest potential show essentials oils from *Eugenia caryophyllus* and *Cinnamomum zeylanicum*.
A. dauci was 100 % inhibited in 0,1 % concentration by EO from *Eugenia caryophyllus*, also by EO from *Cinnamomum zeylanicum.* Other essential oils also shows some inhibition potential, but not in 100 % (*Litsea cubeba*, *Pelargonium graveolens*, *Thymus vulgaris*). Results from field experiments in first year are not so clear and need more research for correct interpretation.

Keywords: fungal pathogens on vegetable, *Alternaria dauci*, carrot, essential oils

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Steaming and biological control: potential strategies for IPM of Fusarium wilt of lettuce?

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Fusarium wilt is an increasing problem in greenhouse-grown lettuce in Belgium and the Netherlands. The disease is caused by the soil-borne fungus *Fusarium oxysporum* f.sp. *lactucae* (fol). Since its first report in Japan in 1955, the disease has been described causing losses in lettuce production areas worldwide. Once the pathogen is established in soil, it is very difficult to manage it. Fol forms resting structures that can survive in soil and plant debris for years. Urgent development of effective control measures is necessary to ensure the future of intensive lettuce cultivation in soil in Belgian and Dutch greenhouses. Commercially available chemical soil fumigants seem not to be able to control the pathogen. In addition, non-chemical control methods are more in line with the IPM approach supported by the European Union. In September 2016, steaming was applied in an infested greenhouse in Belgium. Soil samples were taken from 0-20 cm and 20-40 cm depth before and after steaming. Fusarium populations were determined by plating on Komada's medium. Clear reduction of populations was found in both soil layers after steaming. However, remaining populations were higher in the deepest layer. No disease symptoms were observed during the following two crop cycles. Fusarium populations and crop development will be monitored in the spring and summer of 2017. Use of a biological control agent has shown potential against fol in a pot experiment in the Netherlands, but failed to control the disease in a pot trial in Belgium. Different cultivars, growth conditions and inoculum density probably explain the different results. Currently, more trials are being performed concerning biocontrol.
Keywords: Fusarium oxysporum f.sp. lactucae, IPM, biocontrol, soil disinfestation

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Essential oils on the *Fusarium oxysporum* f.sp. *capsici in vitro* control

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Fusarium wilt on pepper, caused by the fungus *Fusarium oxysporum* f.sp. *capsici*, is an important disease that has caused increasing losses in this crop. This study aimed to evaluate the activity of different essential oils on the mycelial growth and sporulation of *F. oxysporum* f. sp. *capsici*, isolated from sweet peppers. The experiment was carried out in duplicate, on a completely randomized design according to the 8 x 3 factorial scheme [8 essential oils (clove, copaiba, eucalyptus, tea tree, basil, rosemary, citronella and peppermint) x 3 concentrations (0.25% 0.50% and 0.75%)] and one control treatment PDA medium only), with three replications per treatment. The oils were added to the PDA culture medium at the predetermined concentrations. A disc of 0.5 cm diameter of PDA medium with the fungus was placed in the center of each petri dish containing the treatments. The plates were maintained at 25 °C and 12 hour-photoperiod. To evaluate the inhibitory effect of the oils, mycelial growth (diameter of colony) and sporulation (number of spores/mL) for each treatment were determined.In both experiments, citronella, cloves, eucalyptus, peppermint and basil essential oils inhibited completely the mycelial growth, in all concentrations, differing from the control and avoiding the sporulation. For rosemary and copaiba essential oils, high fungal sporulation was observed in both trials, not differing from the control, showing that both essential oils did not present a good inhibitory effect. We can conclude that citronella, clove, eucalyptus, peppermint and basil essential oils can be used for the *Fusarium oxysporum* f.sp. *capsici* alternative control.

Keywords: alternative control, mycelial growth, sporulation, natural fungicide, sustainable development

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COST Action CA16107 EuroXanth: integrating science on *Xanthomonadaceae* for integrated plant disease management in Europe

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Bacteria of the family *Xanthomonadaceae*, including species of *Xanthomonas* and *Xylella fastidiosa*, belong to the most devastating plant pathogens continually challenging food security. Many of the pathogens are listed as quarantine organisms in the EU and their study is of uttermost importance. The concerned pathogens infect all kinds of crop plants, such as cereals, forage crops for ruminant feed, vegetables, fruits, shrubs and trees.
COST (European Cooperation in Science and Technology; www.cost.eu) is a funding agency for research and innovation networks. COST Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation.
This COST Action will bring together some of the brightest and best minds to join in an interdisciplinary network to develop strategies for sustainably protecting plants and significantly reducing yield losses. Specifically, this initiative will address several key aspects of the pathogen-vector-host interactions from the cellular to the population level. A better insight into population structures and virulence mechanisms of the pathogens, together with the exploration of the molecular mechanisms underlying disease resistance to the pathogen, will enable development of durably resistant plant cultivars and exploitation of bio-control schemes tailored to population and pathogen.
This COST Action will generate a platform that gathers experts from different disciplines, such as molecular diagnostics, molecular host-microbe interactions, plant resistance breeding, and applied microbiology. Joining their efforts will help to develop and implement effective plant protection schemes, be it via resistant crop cultivars or via other control mechanisms. This goal will be achieved by mobilizing and training scientists from major European institutions, regulatory bodies and commercial companies working on the various aspects of this complex of problems.
Please follow our COST Action at Twitter (https://twitter.com/EuroXanth), Scoop/it! (http://www.scoop.it/t/xanthomonadaceae-plant-diseases) and ResearchGate (https://www.researchgate.net/project/EuroXanth-Integrating-science-on-Xanthomonadaceae-for-integrated-plant-disease-management-in-Europe).

Keywords: pathogen detection, genetic diversity, population structure, genetic resistance, host defense, disease management

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Enhancing plant disease resistance through synthetic re-engineering of ABA signalling and catabolism

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Using a high resolution, time-resolved microarray dataset of *Pseudomonas syringae* pv. tomato DC3000 infected *Arabidopsis thaliana* leaves we identified early response genes that are specifically targeted by one or more of the 28 DC3000 effector, while remaining unresponsive to abiotic stresses, wounding and a disarmed DC3000*hrp* mutant. We designed strategies to re-engineer hormone signaling pathways utilizing these "effector responsive" promoters to create conditionally activated synthetic constructs which can neutralize pathogen virulence. Here we present results focusing on synthetic constructs designed modulate ABA signaling and ABA catabolism during susceptible interactions. Using modelling informed approaches, we mutated key residues in the PYL5 and PYL9 ABA receptors to enhance binding of pathogen induced ABA without activating the downstream signaling components. Transgenic plants carrying the mutant PYL proteins under the control of the effector responsive promoters showed markedly reduced symptom development and were more resistant to DC3000. Moreover, in re-engineered *PYL9* expressing plants, the levels of ABA responsive transcript, the protein phosphatase 2C, *HAB1*, remained significantly less in comparison to wild type plants at 6 h after DC3000 infection. To validate these results we demonstrate that 35S CaMV over expression the mutated PYL5 generated plants which were more insensitive to exogenous ABA application, indicating that the ABA signaling pathway is disrupted in these mutant PYL5 lines. Concurrently, we generated transgenic lines designed to catabolize pathogen generated ABA driving the ABA catabolic enzyme, CYPA3, under the control of an effector responsive promoter. Transgenic plants carrying the conditionally activated *CYPA3* gene were more resistant to DC3000 infection in comparison to wild type Col-0.

Keywords: ABA, PYL, effector, DC3000, CYPA3

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The BacPlant project: towards a sustainable agriculture by increasing plant tolerance to biotic stress under climatic change

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With 7.27 billion to date, the world population is projected to reach 9.1 billion people by 2050 and the demand for food is already increasing. Meanwhile, the climate is likely to become warmer and wetter during winters and hotter and drier during summers. Climate change (CC) will induce changes in agricultural practices, therefore multiplying the threats to sustainable food security. To counteract, mitigate or control plant diseases, growers often rely heavily on chemical fertilizers and pesticides, but a range of negative impacts for human health and environment. It is therefore crucial to develop relevant and innovative control strategies to protect plants from various diseases. In recent years, promising alternative strategies for improving plant health emerged thanks to the use of beneficial microorganisms (called BCAs for Biological Control Agents) that promote plant growth and immunity. These BCAs can indeed enhance plant resistance and counteract environmental biotic stress. They offer novel possibilities in increasing the sustainability of production systems.
Wheat plays an essential role in societies and their food supply issues. As other crop plants, wheat is subjected to several fungal diseases, which are widely distributed wherever the crop is grown and alter wheat yield and quality. Powdery mildew (PM) and *Septoria tritici* blotch (STB), are among the most prevalent and the most damaging foliar diseases on wheat inducing important yield losses.
With the BacPlant research project, the impact of the climate change (mainly temperature and drought stresses) on the tolerance of wheat to the disease-causing agents responsible for PM and STB in the presence of BCAs will be analyzed.
At a starting point, we used the *Burkholderia phytofirmans* PsJN, a rhizobacterial strain, to develop a colonization protocol. PsJN is an endophytic bacterium which is able to colonize the internal tissues of the plant without causing external signs of infection or negative effects. In our protocol, we inoculated PsJN by soaking grains in bacterial solution for 6 hours. With this method, we found PsJN in aerial parts and in roots several weeks after inoculation using determination on culture media. Bacterial detection by PCR method is currently being performed.
The impact of the presence of PsJN on different traits of the tripartite interaction between wheat, *Burkholderia phytofirmans* and PM/STB will be investigated.

Keywords: biological control agents, climate change, Powdery mildew, *Septoria tritici* blotch, wheat

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Non-leguminous cover crops contribute to *Pratylenchus* control in conservation agriculture

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Soil conservation is one of the major challenges for agriculture in the 21st century emphasizing the need to develop sustainable farming systems based on conservation tillage and permanent soil cover by living and dead plants as well as the use of crop rotations. However, these measures may foster plant-parasitic nematodes with a broad host range (*Pratylenchus* spp.). These are especially problematic on legumes and need to be kept below damage thresholds to ensure ecological services such as biological nitrogen fixation.
The dynamics of the indigenous fauna of plant-parasitic nematodes were studied in eight coordinated Multi-Environment field Experiments (MEEs) under four agro-environmental conditions in Europe (Continental, Nemoral, Atlantic North and Mediterranean North). Ploughed systems were compared with non-inversion tillage and different cropping sequences. The MEEs consisted of a two-year sequence of wheat grown with or without a clover living mulch and followed by leguminous or non-leguminous cover crops or weedy fallow. The second main crops were maize, potatoes, or tomatoes, depending on site.
Wheat grain yields were not affected by up to 900 *Pratylenchus* individuals 100 ml $soil^{-1}$, thus indicating that densities were generally below the economic damage threshold. Initial population densities of *Pratylenchus* were 19-311 nematodes 100 ml $soil^{-1}$ depending on site. They increased by 32% in wheat-maize rotations and decreased by 37% in wheat-potato and wheat-tomato rotations. Final populations were 74% higher under non-inversion tillage compared to ploughing. Leguminous cover crops and living mulches increased the numbers by 89% compared to the weedy fallow. In contrast, oilseed radish (*Raphanus sativus*) and black oat (*Avena strigosa*) cover crops reduced final populations of *Pratylenchus* by 12% compared to the weedy fallow, thus highlighting their value for nematode control particularly for soils with high sand and low humus contents.
The study was supported by the EU 7th Framework program project OSCAR (289277) (www.oscar-covercrops.eu)

Keywords: plant-parasitic nematodes, conservation tillage, crop rotation, subsidiary crops

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Preharvest hormetic doses of UV-C radiation can decrease susceptibility of lettuce to *Botrytis*

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Post harvest applications of UV-C radiation have proven very efficient in reducing the development of post-harvest diseases in many species including lettuce (*Lactuca sativa* L.). Several studies suggest that UV-C radiation is effective not only because of its disinfecting effect but also because it may stimulate plant defences. Preharvest treatment with UV-C radiation may thus offer an interesting potential for lettuce protection, provided that application doses are effective while excluding any harmful effects on the plants. Here we provide evidence that 0.85 $kJ.m^{-2}$ and 1.70 $kJ.m^{-2}$ represent doses of UV-C radiation that are not deleterious for lettuce plants. We used several criteria to evaluate the effect of UV-C radiation on the plant, including histological observations; the concentration of malondialdehyde, an indicator of membrane integrity, as well as parameters derived from measurements of chlorophyll fluorescence, such as maximal efficiency of photosystem II (Fv/Fm) and the Performance Index (PI) of Strasser. We observed that a single dose of 0.85 $kJ.m^{-2}$ slightly increased plant resistance to grey mould (*Botrytis cinerea* L.) while a single dose of 1.70 $kJ.m^{-2}$ had the opposite effect. When a 0.85 $kJ.m^{-2}$ dose was applied 4 times, at two-day intervals, there was an increase in the total phenol content of leaves, and in phenylalanine ammonia lyase, catalase, and MDAHR activities. Leaves inoculated 2 days after the last UV-C treatment showed significantly increased resistance (-30%) when compared to the control.

Keywords: UV-C, *Botrytis*, fluorescence, resistance, PAL, *Lactuca*

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Characterization of plant defense stimulators (PDS) effect according to developmental stage and nitrogen supply

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Plants possess an innate immune system that allows them to defend themselves against pathogens. The use of Plant Defense Stimulators (PDS) is based on this ability of plants to defend against a pathogen. These products can activate different plant defense pathways thereby limiting infection by the pathogens. The use of conventional crop protection products can thus be reduced. The development of SDP capable of eliciting plant defenses is therefore a promising strategy in the current agro-ecological context. The effectiveness of these SDPs in activating systemic defenses depends on several parameters such as the stage of development of the plant and environmental conditions. More specifically, mineral nutrition, and mainly nitrogen nutrition, plays a major role in the establishment of defenses. The response to SDPs also depends on the genotype considered (varietal effect).
The impact of nitrogen and developmental stage on the efficacy of these products was studied in order to identify the optimal conditions for their use. The effects of the stage of development of the plant as well as the rate of nitrate available were studied on *Arabidopsis thaliana* Col0 sprayed with the commercial SDP Bion®.
The activation of defenses in response to SDP was measured by the study of 2 types of responses:
-The level of expression of marker genes of the 2 systemic defense pathways (*PR1* and *PR5* for salicylic acid pathway, *PDF1.2* for jasmonic acid pathway) using qRT-PCR.
-The level of protection against the pathogen *Pseudomonas syringae DC3000* was monitored using counting colony unit.
The results of these two interrelated analyzes will be presented at this seminar.

Keywords: plant defense stimulator, nitrogen, stage, plant pathogen

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Effect of seed treatment with novel strains of *Trichoderma* spp. on establishment and yield of spring wheat

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Fusarium head blight (FHB) is the most important disease of wheat in Canada. FHB reduces grain yield and quality and results in seed contamination with *Fusarium* spp. that is associated with reduced seed vigor and poor stand establishment in wheat. The effect of seed treatments with six strains representing three species *Trichoderma*, selected based on their superior antagonistic ability on mycelium growth of *F. graminearum* in dual culture assays, on wheat seed lots contaminated with *Fusarium* spp. (28-43%) was examined in field trials in 2008, 2009, and 2011. None of the six strains of *Trichoderma* spp. showed a significant seed treatment effect for all parameters measured each year, but over the three years, all six strains significantly reduced root rot severity and increased yield, three stains (Trich12, TrichC70 and TrichPine) increased emergence and four strains (Trich06, TrichC39, TrichC70, and TrichMM7) increased plant dry weight, compared with the untreated control. TrichC70 was the only strain that showed a significant improvement to all four parameters, increasing emergence by 10.9%, dry weight by 51.7%, and yield by 11.0% and reducing root rot severity by 51.7%. These effects were less but not significantly different from that of the registered fungicide Vitaflo-280 (carbathiin + thiram) used as the positive control in the field trials. The results indicate that *Trichoderma* stain TrichC70 may be used as an alternative to fungicide seed treatments to alleviate the detrimental effect of the seed-borne phase of FHB in wheat.

Keywords: *Fusarium graminearum, Trichoderma* spp., biological control, seed treatment, spring wheat

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Chemical screen of natural small molecules identified a steroidal alkaloid, holaphyllamine, able to induce defense responses in *Arabidopsis thaliana* and increase resistance against *Pseudomonas syringae*

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Plants rely on their innate immune system to combat microbial infections. Using a chemical screen of 1,600 plant-derived compounds, we have identified a steroidal alkaloid that is able to induce defense responses in *Arabidopsis thaliana* without altering growth. The identified alkaloid is holaphyllamine (HPA) whose chemical structures is similar to steroid pregnanes of mammals. While HPA was not detected in untreated Arabidopsis seedlings, the steroidal alkaloid is able to trigger the formation of reactive oxygen species, accumulation of callose and expression of a number of pathogenesis-related genes of the salicylic and jasmonic acid pathways. In addition, we show that pre-treatment of Arabidopsis seedlings with HPA before infection with the pathogenic bacterium *Pseudomonas syringae DC 3000* results on a significant reduction of symptoms (i.e., reduction of necrosis and preservation of pigment content). Our findings demonstrate that plant-derived HPA is able to activate the plant immune system and improve resistance against pathogenic bacterial infection.

Keywords: *Arabidopsis thaliana*, chemical screen, holaphyllamine, plant defense, *Pseudomonas syringae*, steroidal alkaloids

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The effect of fungicides and plant growth regulators application on the severity of Phoma stem canker and seed yield of winter oilseed rape

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At present study, multifactorial field experiments have been conducted over three growing seasons (2013 to 2015) to evaluate the effect of five fungicides and plant growth regulators application on growth parameters, yield parameters and seed yield of four different winter oilseed rape varieties. Cultivars Elektra, Genie, PR 46W20 and Vitara were chosen because of their differences in resistance to winter hardiness, lodging and blackleg disease caused by *Leptosphaeria maculans*. The experimental design was a completely randomized block design, comprising four replications for each plant variety per treatment for each growing season. Fungicides and plant growth regulators (Ampera: prochloraz and tebuconazole; Carax: mepiquat and metconazole; Folicur: tebuconazole; Tilmor: tebuconazole and prothioconazole and a mixture of Imbrex plus Folicur: fluxapyroxad plus tebuconazole) were applied twice; in autumn (BBCH 14-18) and in spring (BBCH 30-55).
Results showed that fungicide and plant growth application affected oilseed rape growth and yield parameters during growing season, but effect depended on meteorological conditions in research year, as well as used oilseed rape varieties. Plant growth regulators had significant effects on plant height and winter killing (%). Plots treated with Carax had the shortest height and plots treated with Folicur had the lowest mortality rate of plants after winter. Significant differences in *L. maculans*-disease severity were observed among treatment and the varieties. Disease severity was the highest in oilseed rape cv. PR 46W20. The fungicide mixture Imbrex plus Folicur decreased significantly the stem canker development on stem bases than that of those from untreated control and other treatments. Thousand grain weight (g) and yield (t/ha) of oilseed rape varieties were improved significantly when Tilmor or a mixture of Imbrex plus Folicur were applied.

Keywords: *Brassica napus*, winter hardiness, lodging, Leptosphaeria maculans, disease severity, winter hardiness

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Bioinformatics tools dedicated to secondary metabolite analysis: contribution to the screening platform of Bioscreen Interreg Va project

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Bioscreen is one of the fifth constitutive projects of the SmartBioControl portfolio, granted by Interreg Va program. The aim of this project is to build a platform to enhance the screening for biocontrol agents. The bioinformatics approach will be presented, describing the diverse tools developed by University of Lille-Sciences and Technologies partner, dedicated to nonribosomal analysis. Their use to predict bioproduction of secondary metabolites with biocontrol applications, and their contribution to the efficient screening within the platform will be explained, supported by relevant examples.

Keywords: bioinformatics, Norine, Interreg, SmartBioControl, bioscreen

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BioProtect; Biological crop protection in practice: optimization of the efficacy of biological crop protection products in (field) trials

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The use of biocontrol products to combat or to control pests and diseases plays an important role in both organic and conventional farming. The InterregV project BioProtect which is part of Smart Biocontrol, the European Platform of research and development of tools for biocontrol of phytopathogens, contributes to this. Currently, biocontrol products often provide variable efficacy results or insufficient efficacy in comparison to chemical pesticides. For this reason, growers are not convinced to use biopesticides.
Therefore, the project aims to gain more insight in the mode of action of some biocontrol products through a literature study, an agricultural practice survey and (field) trials. Experiments in the field as well as small scale tests under more controlled conditions will be conducted focusing on pathosystems that are of great importance for the cross border region France-Wallonia-Flanders. Not only recognized biological crop protection products, but also new biocontrol products that are currently under development, will be included. After determination of the optimal application timing, application method and application techniques, this knowledge will be transferred to the farmers and growers on the basis of demonstration platforms.

Keywords: biocontrol products, biological crop protection, disease management, smartbiocontrol

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Alternative methods for crops protection: interest on natural substances, microorganisms and plants

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FREDON Nord Pas-de-Calais known as "Organisme à Vocation Sanitaire (OVS)/ Plant Health Organisation" in plant production is specialist in crop protection. Within of her activities of "Research and development", FREDON develops and shares about alternative methods such as natural substances and microorganisms having repulsive effect or straight influence on bioagresseurs to major crops in North of France. Also, FREDON evaluate different plants producing volatile organic compounds (VOC) with a repulsive effect on insects.
Some references were learned about these particular targets:

- Chicory/wireworms,
- Chrysanthemum/thrips,
- Wheat/common bunt,
- Leek/ fly and thrips,
- Potatoes/late blight, wireworms,
- Apple tree/apple scab, apple sawfly, apple weevil, aphids,
- Speedwell/fusarium wilt.

Natural substances that have been evaluated in controlled conditions (in Clinic of the plant (Clinique du Végétal®) at FREDON) and/or on field trials and/or on field productions are : essentials oils, lipopeptides, basic substances and plants extract such as, for example, decoction and infusion. Microorganisms test are made with entomopathogenic fungus antagonist or stimulating of plant health.
Starting new project like Bioprotect (within the project portfolio SMARTBIOCONTROL) give us prospects for improve evaluation skills with Interreg V Flanders and Wallonia cross-border collaboration.

Keywords: natural substances, microorganisms, biocontrol, crops, protection

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BIOPROD FWVL Interreg Project: New strategies for production and formulation of low toxicity biopesticides

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In the context of more respectful utilisation of pesticides in the environment, lipopeptides are very promising molecules. The principal objectives of the FWVL Interreg BIOPROD project are to remove the constraints related to the industrial production of these new bio-pesticides and the microorganisms that produce them. The multi-disciplinary and collaborative works will be conducted on both sides of the border by operators specialized in their respective fields. Thus, the actions conducted in this project will be:

•Optimisation of production and purification conditions of molecules by developing, among others, new innovative procedures implementing high-throughput screening methods

•Sizing and scaling up of installations to achieve future industrial production of lipopeptides These two technical approaches will contribute to determination of a cost for each molecule, which will be one of the fundamentals for the market analysis hereafter mentioned.

•Detailed studies of biodegradability and toxicity of molecules on different models in order to demonstrate the positive impact of these new bio-pesticides compared with the chemicals currently used in agriculture.

•Realisation of many formulations tries in order to get these molecules commercialized, more stable, more active and easier in utilization. These different actions will be complemented by a cross-border market study to determine the practices and expectations of phytosanitary distributors and farmers. The results will be compared in order to have a territorially adapted communication aimed at promoting these new phytosanitary molecules. The ultimate result of the project being the placing on the market in a close future of these products and thus improve the protection of environment through the use of respectful new bio-pesticides that are more respectful for nature and human.

Keywords: biopesticides, high throughput screening methods, innovative process

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Fungi as biocontrol agents: screening strategy towards various plant pathogens - promising results on potato plants challenged by *Phytophthora infestans*

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Potato plants are facing numerous pathogens. Among these is *Phytophthora infestans*, the causal agent of late blight. Currently, chemical treatments are the most recommended strategies for the control of this disease, despite their detrimental impacts on environment and human health. The development of alternative strategies is thus becoming essential for reducing/controlling pesticides applications.

In that context, the use of fungi and fungal bio-effectors represents a promising strategy to control, at least partially, below and above-ground pathogens, and the screening of collections opens the perspective of discovering new environmental-friendly actives molecules.

The main objective of our research was to develop an efficient screening method to identify efficient fungal strains for the biocontrol of *P. infestans* and alternatively of other fungal diseases.

One hundred fifty fungal strains were first selected from the literature for their potential as biocontrol agent. These strains were provided by the Mycothèque of the Université catholique de Louvain (BCCM/MUCL), one of the world largest collection of fungi of agro-food and environmental interest. A 96-well microplate *in vitro* test was used to screen crude extracts of the fungal collection. Sixty-nine organisms (i.e. crude extracts) displayed a strong inhibitory on the growth of *P. infestans*. Their potential activity was then evaluated *in vitro* on potato plantlets grown in microboxes. Seventeen strains showed a strong activity against *P. infestans*. These strains mostly belong to *Aspergillus*, *Chaetomium*, *Fusarium*, *Hyphomyces*, *Penicillium* and *Trichoderma* species. Further isolation, purification and characterization of the active molecules are on-going.

This screening strategy allows the fast track identification of fungi as well as fungal bio-effectors effective against *P. infestans* and will be extended to other major root and shoot pathogens in the SMARTBIOCONTROL project.

Keywords: fungi, screening, biocontrol agents, *Phytophthora infestans*, potato late blight, microplate *in vitro* test

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Mosaics of plant disease resistance genes are a more versatile means of achieving disease control than pyramids in most agricultural landscapes

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The breakdown of plant virus resistance genes is a major issue in agriculture. We investigated whether a set of resistance genes would last longer when stacked into a single plant cultivar (pyramiding) or when deployed individually in regional mosaics (mosaic strategy).

We modeled the genetic and epidemiological processes shaping the demo-genetic dynamics of viruses under a multi-locus gene-for-gene system, from the plant to landscape scales. The landscape consisted of many fields, was subject to seasonality, and of a reservoir hosting viruses year-round.

Strategy performance depended principally on the fitness costs of adaptive mutations, epidemic intensity before resistance deployment and landscape connectivity. Mosaics were at least as good as pyramiding strategies in most production situations tested. Pyramiding strategies performed better only with slowly changing virus reservoir dynamics. Mosaics are more versatile than pyramiding strategies, and we found that deploying a mosaic of three to five resistance genes generally provided effective disease control, unless the epidemics were driven mostly by within-field infections.

We considered the epidemiological and evolutionary mechanisms underlying the greater versatility of mosaics in our case study, with a view to providing breeders and growers with guidance as to the most appropriate deployment strategy.

Keywords: Durable disease resistance, Pyramids of plant resistance, Mosaic of plant resistance, Landscape epidemiology, Regional deployment strategy