Studies of the population structure of the arbuscular mycorrhiza fungus Rhizophagus irregularis

Marine Peyret Guzzon, Charlène Mansuy, Herbert Stockinger, Dirk Redecker

Abstracts of the Third annual joint meeting on Plant Microbe Interactions

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Burgundy Regional Council
Dijon, France

Program:

8h20-8h30: Opening
Mr Daniel Wipf, UMR Agroécologie, Pôle IPM
Mr Jacques Rebillard - Representive of the Burgundy Regional Council

8h30-9h00
Mr. Christian Seebode, Generalkonsul der Bundesrepublik Deutschland
Wissen und Wissensmanagement

9h00-10h30 **session 1:**
Plant-Microbe Interactions: mechanisms and signaling

10h30-11h00 coffee break

11h00-12h30 **session 1:**
Plant-Microbe Interactions: mechanisms and signaling

12h30-14h00 lunch

14h00-15h30 **session 1:**
Plant-Microbe Interactions: mechanisms and signaling
session 2:
Fungal diversity in plant-microbe interactions

15h30-16h00 coffee break

16h00-18h00 **session 2:**
Fungal diversity in plant-microbe interactions
session 3:
Plant-Microbe Interactions: applied research

TALKS of 10 minutes+5 minutes for questions

Abstracts

SESSION 1; PLANT-MICROBE INTERACTIONS: MECHANISMS AND SIGNALING

9h00-9h15: Label-free 1-DE-LC-MS/MS to identify arbuscular mycorrhiza related membrane proteins.

Cosette Abdallah[1,2], Jenny Renaut[1], Benoît Valot[3], Michel Zivy[3], Ghislaine Recorbet[2], Daniel Wipf[2], Eliane Dumas-Gaudot[2].
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Deep changes in the shape and number of organelles, together with profound modifications in various membrane compartments, are induced within arbuscular mycorrhizal (AM) symbiosis. In this context, to investigate the membrane-associated proteins that are regulated in the model interaction *Medicago truncatula* – *Rhizophagus irregularis*, label-free 1DE-LC-MS/MS approach has been employed as alternative to two-dimensional gel electrophoresis. The existence of a correlation between protein abundance and peak areas or number of MS/MS spectra has widened the choice of label-free quantitative proteomics. The results highlighted microsomal protein candidates that could be involved in the symbiotic exchanges between plant and fungal cells.

9h15-9h30: Development of an artificial miRNA system for analyzing gene function in mycorrhizal *Medicago truncatula* roots using the endogenous miRNA159b precursor

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It is a common strategy for the functional analysis of gene products to knock out the corresponding gene and to analyze the resulting phenotype. However, knock out mutants for the gene of interest, like T-DNA or transposon insertion as well as deletions produced by fast neutron bombardment are not always available. In these cases scientists make use of the RNA interference (RNAi) or viral induced gene silencing (VIGS) to produce knock-down mutants. No VIGS system has been established for *M. truncatula* so far, so only the RNAi system remains and is extensively used together with *Agrobacterium rhizogenes* mediated root transformation for functional gene analysis in labs working with *M. truncatula*. The RNAi is based on a hairpin construct with short inverted fragments of the gene of interest. The expressed RNA folds into a perfect matched double strand and is processed by the DCL proteins to short interfering RNAs (siRNAs). Sometimes this

single approach is limited by inefficient knock-down of the gene of interest due to unknown reasons. Additionally, the RNAi system produces a heterogeneous accumulation of siRNAs produced from the hairpin which could lead to unspecific down regulation especially in large gene families with high sequence homology. Therefore, an alternative approach is desired.
An alternative approach using artificial micro RNAs (amiRs) was first developed in *Arabidopsis thaliana* and allows a specific knock down of the gene of interest due to the production of a single 21nt small RNA species. This system has been successfully adapted to *Oryza sativa*, *Physcomitrella patens* and *Chlamydomonas reinhardtii* using species specific miRNA backbones.
Here we demonstrate that mtr-MIR159b extremely precise and defined processed and thus a capable backbone for the use as an amiR system in *M. truncatula*. The amiR is produced by an easy PCR strategy analogue to the *A. thaliana* system and efficient knock-down of target genes could be validated by an amiR against DsRed using the mtr-MIR159b backbone.

9h30-9h45: Regulation of *Rhizophagus irregularis* gene expression in response to strigolactones

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Strigolactones are trace exuded plant signaling molecules perceived by Arbuscular Mycorrhizal Fungi (AMF), triggering fungal cell changes that prepare symbiosis establishment. These molecules induce modifications in the mitochondriome (mitochondrial metabolim and shape) and hyphal branching (invasive growth) on *Gigaspora* species (Besserer *et al*., 2006). We here defined an original experimental design that allowed observing and monitoring strigolactone-induced branching on the AMF model *Rhizophagus irregularis*. Strigolactones also rapidly modified mitochondria shape (within an hour).

As these morphological changes might be accompanied by gene expression regulation, strigolactone effects on *R. irregularis* transcriptome were tested. We performed a MiSeq on GR24 (a strigolactone synthetic analog) treated spores. cDNAs of 250bp were sequenced resulting in 14 million reads of 150b pair end, generating 2.1Gb. Pair ends were first de novo assembled and then the resulting contigs were used for expression analysis. More than 40 fungal genes were found to be differentially expressed in response to strigolactone. For example, a chitin synthase, potentially involved in hyphal growth, was overexpressed 30 times in response to GR24. This experiment confirmed that strigolactones modulate fungal gene expression and justifies the development of an exhaustive transcriptomic analysis.

Besserer A *et al*.. 2006. *PloS Biology* 4: 1239-1247.

9h45-10h00: The plant resistance inducer β-aminobutyric acid (BABA) induces an iron deficiency response in *A. thaliana*

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β-aminobutyric acid (BABA) is a well-known plant resistance inducer. However, the molecular mechanisms underlying its effects are poorly understood. In the present study, we investigated whether BABA could act through the modification of iron homeostasis in *Arabidopsis thaliana*. Supporting this assumption, we obtained first evidences that BABA chelates iron with high affinity. We showed that pre-treatment of plants with BABA induced a drastic but transient iron deficiency response. Quantification of iron indicated that this response is related to the perturbation of iron distribution/availability rather than a reduction of iron assimilation. Finally, we provided evidence that the iron deficiency response triggered by BABA could be one of the determinants of its protective effects against *Botrytis cinerea*.

10h00-10h15: MtGPAT, a glycerol-phosphate-acyltransferase, essential for arbuscular mycorrhizal symbiosis in *Medicago truncatula*

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The establishment of arbuscular mycorrhizal (AM) symbiosis is accompanied by the formation of specialized fungal structures, the arbuscules, in the cortical root cells of the plant host. The development of these fungal structures within the host cells affects cell morphology and results in drastic membrane reorganization events. Therefore, the reconstruction of plant cell membranes presumably requires large amounts of newly synthesized fatty acids and lipids. This includes the breakdown of storage lipids and enhanced *de novo* synthesis.
However, the present study clearly indicates remodelling of the cellular membrane system supported by strong alterations in the membrane lipid composition of mycorrhizal roots. Furthermore, the colonization of host cells resulted in the upregulation of transcripts related to lipid metabolism. Similar strongly enhanced expression levels of genes encoding for enzymes involved in lipid metabolism were found in cells adjacent to arbuscule containing cells. We found a transcript encoding a glycerol-3-phosphate acyltransferase (GPAT) that had a highly elevated expression in arbuscule-containing cells. This enzyme participates in the glycerolipid- and glycerophospholipid metabolism. Seven GPAT family members are known in *Medicago truncatula*, but only *MtGPAT1* is specifically expressed in mycorrhizal roots.
The objective of current studies is the functional characterization of MtGPAT1 by molecular and biochemical phenotyping of transformants to elucidate its role during AM symbiosis and the concomitant maintenance of the periarbuscular membrane.

In general, we expect new insights into the relationship between lipid metabolism and AM symbiosis, since glycerolipids are indispensable for cell membrane composition, while also being components of several signaling processes.

10h15-10h30: *MtGras8* as a link between phosphate homeostasis and arbuscule development during arbuscular mycorrhizal symbiosis in *Medicago truncatula*

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During arbuscular mycorrhizal (AM) and root nodule symbiosis GRAS transcription factors (TFs), such as Nodulation Signaling Pathway 1 and 2 (NSP1 and NSP2), play key roles in signal transduction downstream of *DMI3* (Maillet et al., 2011) A cell type specific transcriptomic data set of *G. intraradices* inoculated *Medicago truncatula* roots was obtained by combining Laser Capture Microdissection and Affymetrix®GeneChip hybridization. In this data set, a member of the GRAS TF family, *MtGras8,* was exclusively induced in arbuscule-containing cells. This cell type specific expression could be confirmed *via* promoter*::uidA* fusion studies and cell type specific qRT-PCR analysis. RNA interference driven by the *MtPt4*-promoter enabled an arbuscule-containing cell specific gene silencing of *MtGras8*. *MtPt4pro::MtGras8*-RNAi roots showed a significantly reduced colonization, and an elevated number of degenerated arbuscules were observed.
Furthermore, a deep sequencing analysis of small RNAs followed by stem-loop qRT-PCR revealed that *MtGras8* is a target of microRNA5204* (Devers et al., 2011). Since miR5204* is induced by low phosphate, we assume that *MtGras8* might represent a link between phosphate homeostasis and arbuscule development. The role of the miR5204*-based cleavage of *MtGras8* transcripts will be analyzed *via* overexpression of miR5204*. In addition a misregulation of the miRNA-based cleavage will be achieved by expression of *MtGras8* including a mutated microRNA5204*-cleavage site.

11h00-11h15: Identification of sugar transporters in arbuscular mycorrhiza

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Our study focuses on sugar transporters from both plant and fungal partners at the symbiotic interface to better understand biotrophic exchange systems. Thereby, a collection of putative contigs and ESTs of hexose transporters from *Medicago truncatula* and *Fragaria x ananassa* will be processed. The full length sequences will be cloned for functional complementation and uptake experiments in transport deficient yeast mutants.

This work also investigates the influence of different mycorrhizal fungi on (1) the expression level of sugar transporters and (2) the economically relevant part of *F. x ananassa* by analyzing its impact on the plant and the fruit. To this aim, combination of phenological observations, transcriptomics (qRT-PCR and Microarrays) and biochemical analyses will be performed.
The need of the complete characterization of hexose transporters is reinforced by the recent demonstration of enhanced sugar content in mycorrhizal strawberries (G. Berta and G. Lingua; personal communication).

11h15-11h30: Expression of Sorghum bicolor ammonium transporters upon colonization with arbuscular mycorrhizal fungi

Sally Koegel*, Nassima Ait Lahmidi**, Christine Arnould**, Florian Walder*, Kurt Ineichen*, Thomas Boller*, Daniel Wipf**, Andres Wiemken* and Pierre-Emmanuel Courty*
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Arbuscular mycorrhizal fungi (AMF) are important plant symbionts, trading mineral nutrients beyond the reach of roots, in particular ammonium, in exchange to their host's photosynthetic products. Sorghum bicolor is one of the world's leading cereal crops, providing food, fibre and fuel across a range of environments and production systems. It has a particular ability to be productive even under strongly adverse conditions, tolerating much more severe drought than most other grain crops. As its genome has recently been sequenced, we have characterized all eight members of the ammonium transporter (AMT) family and studied their expression in different tissues of field-grown plants. While most of them were well expressed in all the tissues, SbAMT3;1 was predominantly and SbAMT4 was exclusively expressed in roots. Both were highly up-regulated (up to 70 and 20 times, respectively) in the presence of AMF. A split-root and a laser microdissection experiment have shown that SbAMT3;1 and SbAMT4 were locally but not systemically induced around arbuscules. Immunolocalization has indicated that the protein SbAMT3;1 was co-localized with mature arbuscules indicating a possible important role of this transporter in the AMF symbiosis.

11h30-11h45: Transcriptional response of *Medicago truncatula* sulphate transporters to arbuscular mycorrhizal symbiosis with and without sulphur stress

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Sulphur is an essential macronutrient for plant growth, development, and response to various abiotic and biotic stresses due to its key role in the biosynthesis of many S-containing compounds. Sulphate represents a very small portion of soil S pull and it's

the only form that plant roots can uptake and mobilize through H+-dependent co-transport processes implying sulphate transporters. Unlike the other organically bound forms of S, sulphate is normally leached from soils due to its solubility in water, thus reducing its availability to plants.
Although our knowledge of plant sulphate transporters has been growing significantly in the last decades, little is still known about the effect of the arbuscular mycorrhiza interaction on sulphur uptake. Carbon, nitrogen and sulphur measurements in plant parts and expression analysis of genes encoding putative Medicago sulphate transporters (MtSULTRs) were performed to better understand the beneficial effects of mycorrhizal interaction on Medicago truncatula plants colonized by Glomus intraradices at different sulphate concentrations. Mycorrhization significantly promoted plant growth and sulphur content, suggesting increased sulphate absorption. In-silico analyses allowed identifying 8 putative MtSULTRs phylogenetically distributed over the 4 sulphate transporter groups. Some putative MtSULTRs were transcribed differentially in roots and leaves and affected by sulphate concentration, while others were more constitutively transcribed. Mycorrhizal-inducible and -repressed MtSULTRs transcripts were identified allowing to shed light on the role of mycorrhizal interaction in sulphate uptake.

11h45-12h00: The arbuscular mycorrhizal symbiosis influences sulfur starvation responses of *Medicago truncatula*

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Beside phosphate, nitrate and ammonium, sulfur compounds are also symbiotically transferred from AM fungus to host plants, however, the physiological importance of mycorrhizal-mediated sulfur on the plant metabolism has not yet been determined. Here we analyze the impact of a mycorrhizal colonization on sulfur stress responses in *Medicago truncatula*.
We applied different sulfur and phosphate fertilization treatments to *M. truncatula* and analyzed whether a mycorrhizal colonization influences leaf metabolite composition and the expression of sulfur starvation-related genes.
Expression pattern of sulfur starvation-related genes indicated reduced sulfur starvation responses in mycorrhizal plants grown under moderate phosphate nutrition. Leaf metabolite levels clearly showed that phosphate stress is of superior impact for the plant metabolism with no demand for sulfur at strong phosphate starvation. However, under moderate phosphate stress, mycorrhizal colonization reduces sulfur stress responses, likely due to the symbiotic sulfur uptake.

12h00-12h15: A putative link between sucrose transport and brassinosteroid signaling?

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Regulation of sucrose transport and sucrose transporter expression has intensively investigated during the last decades and recent advances in the identification of protein-protein interactions of plant sucrose transporters helped to identify new candidate genes involved in sucrose transporter regulation, stability, secretion and targeting. Sucrose transporters were shown to undergo endocytosis and to be recycled at the plasma membrane.
Several recent publications suggest a potential impact of brassinosteroids on photoassi milate partitioning and sugar content in higher plants. Using the yeast two hybrid split ubiquitin system, we identified several candidates interacting with the sucrose transporter SlSUT2 from tomato. Three of them seem to be related to brassinosteroid signaling and /or biosynthesis. One of the SUT2-interacting proteins, MSBP1, is assumed to negatively affect brassinosteroid signaling via endocytosis of BAK1, the brassinosteroid co-receptor. Recently, involvement of MSBP1 in mycorrhization of *Medicago truncatula* has been published by other groups. The role of MSBP1 as well as SlSUT2 for mycorrhization of phosphate starved tomato plants will be analyzed in detail.

12h15-12h30: Gas exchange dynamics of shoot and root zone in mycorrhizal tomato plants

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The symbiosis between arbuscular mycorrhiza (AM) and most vascular plants is a common and worldwide occurring, often beneficial association between fungi of the phylum Glomeromycota and most land plants. One of the effects of the AM symbiosis can be enhanced photosynthetic activity of the host plant which balances the additional demand of the fungal root colonizer for photoassimilates. The reasons for these changes can be various and are discussed extensively in literature such as the phosphate status, water availability, sink metabolism or hormonal changes of the host. Recently, we showed that enhanced photosynthetic activity in mycorrhizal tomato plants occurred without changes in the plant phosphate status and accompanied by transcriptional up-regulation of sucrose transporter genes (*LeSUT*). In order to get more insight into the carbon relations of the plant-fungal symbiosis, gas exchange measurements of the shoot and the root zone were simultaneously carried out over all stages of the interaction. Our experiments have shown that the increased respiratory activity of the root zone of mycorrhizal tomato plants is delayed compared to the enhanced photosynthetic activity. Additionally, changes in the colonization patterns of the host roots by AM fungi were observed, when SUT expression is inhibited in the host.

14h00-14h15: Identification of *in planta* expressed *Glomus intraradices* sugar transporter genes

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The nutrient exchange between the plant and the fungal partner of the arbuscular mycorrhizal (AM) symbiosis is the key element of this mutual interaction. So far only little is known about fungal proteins involved in transport processes in arbuscule-containing cells. This is mainly due to the obligate biotrophy of AM fungi, the difficulty to collect fungal material for downstream analyses and the yet not fully known genome sequence.
To get more insight into the fungal side of the symbiotic interaction, we used Laser Capture Microdissection (LCM)-mediated harvesting of *G. intraradices* arbuscules and sequencing by synthesis (SBS) sequencing. We identified a large number of fungal genes that are differentially expressed in arbuscules compared to the extraradical mycelium. Several of the genes upregulated in arbuscules are putative sugar transporters, and therefore likely to be involved in the sugar uptake of the fungus from the symbiotic interface. We are currently characterizing several of these putative fungal sugar transporters.

14h15-14h30: Phosphatidic acid signalling in cryptogein-elicited tobacco cells

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Phosphatidic acid (PA) is a conserved phopholipid second messenger involved in stress response, metabolism and development in animals and plants. PA can be either generated by phospholipase D-mediated hydrolysis of phospholipids or produced by the sequential action of phospholipase C (PLC) and diacylglycerol kinase (DGK). Biochemical and pharmacological approaches carried in our laboratory indicate that a PLC/DGK pathway is activated in tobacco cell culture elicited with the oomycetal protein cryptogein. These early signalling events taking place within the first 30 minutes following the elicitation are likely to locate in plasma membrane rafts, i.e. membrane compartment highly enriched in sterols and sphingolipids that serves as signalling platformes. PA originating from the cryptogein-induced PLC/DGK pathway is further believed to control oxidative burst through regulation of the raft-localized NADPH oxidase.

14h30-14h45: Early responses of *Medicago truncatula* roots after contact to *Rhizophagus irregularis*

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The plant mechanisms used to establish a molecular dialog with arbuscular mycorrhizal fungi (AMF) is of crucial importance for targeted implementation of the symbiosis in plant production. It is hypothesized, that plants undergo more changes in the presymbiotic phase apart from the exudation of strigolactones to attract their symbiotic partner. The very first reaction of the plant towards the presence of AMF will be analyzed on basis of transcriptional changes, secondary metabolites in the root tissue as well as secondary metabolites in root exudates of *Medicago truncatula* cv Jemalong A17 in contact with *Rhizophagus irregularis*. The transcriptional differences will be captured using a microarray. For the analysis of secondary metabolites in roots and root exudates, the methods of untargeted LC-MS analysis as well as SPME for volatile analysis have been chosen. First analyses revealed some differences between exudate patterns of plant roots compared to roots subjected to AM fungal material.

SESSION 2; FUNGAL DIVERSITY IN PLANT-MICROBE INTERACTIONS

14h45-15h00: Diversity of arbuscular mycorrhizal fungi (*Glomeromycota*) in european soils analysed by pyrosequencing

Bouffaud M-L, Stockinger H, Peyret-Guzzon M, van Tuinen D, Wipf D, Redecker D
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Arbuscular mycorrhiza provides essential ecosystem functions in natural and human-dominated ecosystems. Generally human activities like agriculture seem to have a negative effect on diversity of arbuscular mycorrhizal fungi (AMF), and thus on ecosystem functioning. New sequencing technologies now allow to assess AMF diversity on a much larger scale than previously. In the context of the European project EcoFINDERS, five Long-Term Observatories across Europe with different soil management have been studied. The diversity of AMF in these soils is analyzed by pyrosequencing, using the ITS (rDNA Internal Transcribed Spacers) as marker, which were recently determined as the standard barcoding gene for the fungi. The possibility to use other genes as alternative markers was also explored.

15h00-15h15: Studies of the population structure of the arbuscular mycorrhiza fungus *Rhizophagus irregularis*

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The aim of this project is to better understand biodiversity of arbuscular mycorrhiza (AM) fungi at the community and population levels. Populations of AM fungi are studied in different habitats at several scales under the impact of disturbance and fertilization (phosphorus or/and nitrogen). The influence of these factors was addressed in a field experiment. This study focuses on *Rhizophagus irregularis*, the widespread model species, previously named *Glomus intraradices*. Competition between strains of this species was assessed under controlled conditions in a growth chamber experiment using mitochondrial large subunit of ribosomal DNA as a specific marker for Real Time PCR.

15h15-15h30: First identification of polymorphic microsatellite markers in the Burgundy truffle, *Tuber aestivum* (Tuberaceae)

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Tuber aestivum, the most common truffle in Europe, tends to take an important place in the truffle economic market. For the first time, microsatellites primers were developed to investigate the polymorphism within this species. Using direct shotgun pyrosequencing, 15 polymorphic microsatellites were identified out of the 7,784 perfect microsatellites present in the 534,620 reads obtained. Tested on 75 samples, these microsatellites were highly polymorphic. Alleles varied from 4 to 15 and the expected heterozygosity from 0.266 to 0.620. A multi-locus analysis allowed the identification of 63 genotypes over the 75 samples analyzed. Direct shotgun pyrosequencing is a fast and relatively low cost technique allowing identification of microsatellites in non-model species. The microsatellites developed here will allow performing a genetic population study to infer the evolutionary history of this species.

16h00-16h15: Impact of deoxynivalenol on soil microflora and fauna

Muhammad Abid, Léon Fayolle, Elodie Gautheron, Cécile Heraud, Nadine Gautheron, Véronique Edel-Hermann and Christian Steinberg
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F. graminearum is an important pathogen that causes head blight of cereal crops as wheat and maize. It also produces the mycotoxins (as Deoxynivalenol=DON) which are toxic to the human and animals. During the off season the pathogen survives in the soil, on weeds and in crop residues. A 24 weeks study was conducted in controlled conditions (microcosms of natural soil, 17 °C, 80% WHC) to test whether the presence of DON in the wheat crop residues gives competitive advantage to *F. graminearum* over the other soil microflora and fauna to survive and develop a primary inoculum during the decomposition process. This study was carried out in the presence of the whole soil biota (i.e. fungi, bacteria, protozoa, nematodes and earthworms). In this experimental approach, wheat straw was inoculated with *F.*

graminearum. The latter was placed on the soil surface or incorporated into the natural soil. This experiment was conducted with (1mg DON/kg soil-straw mixture) and without DON. The molecular biomass of fungi, bacteria and *F. graminearum* was determined by qPCR (real time polymerase chain reaction). The changes in the community structure of fungi, bacteria, protozoa and nematodes were determined by T-RFLP (terminal restriction fragment length polymorphism). The results suggested that DON in wheat straw showed an impact on part of the biotic components of the soil but the impact depended on the communities and on the location of the wheat residues.

16h15-16h30: Search for indicators of soil suppressiveness to soil-borne diseases: functional genomics approach.

Katarzyna Siegel,[1] S. Aimé,[1] J. Raaijmaakers,[2] W. Deboer,[3] P. Lemanceau,[1] C. Steinberg[1].
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Soils which are suppressive to soil-borne diseases are soils in which pathogens, although present, sometimes in large inoculum density, cannot carry their infectious activity. In most cases, biotic factors are responsible for this inhibition, although the abiotic environment regulates these factors.
In this thesis, we propose to conduct an intermediate approach between the metagenomic analysis of soil suppressiveness to diseases and the search for fungi whose activities contribute to the inhibition of infectious pathogens. The aim is to define a set of fungal genes a priori associated with mechanisms reflecting the suppressiveness of given soils to a disease and to check whether these genes are common to all the suppressive soils to different types of disease regardless of the taxonomic biodiversity present in these soils. Bioassays were conducted in both suppressive and conducive soils, DNA was extracted from the rhizosphere of susceptible plants grown in these soils, 454 sequencing of prokaryotic and eukaryotic DNA is in progress and tools to manage the data are being setting up in the frame of the UE project Ecofinders FP7-ENV-2010-264465.

SESSION 3; PLANT-MICROBE INTERACTIONS: APPLIED RESEARCH

16h30-16h45: Transcriptomes of compatible and non-compatible gray mold / grapevine interactions compared in search of resistance factors

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Gray mold (*Botrytis cinerea*) causes significant annual yield and quality losses to viticulture. Comparison of transcriptomes of compatible and non-compatible

interaction with *Vitis vinifera* cv Marselan – on harvesting stage berries or véraison stage berries, respectively – suggest the jasmonic acid pathway is activated in the former, while the salicylic acid pathway is more pronounced in the latter. In addition, in the non-compatible case, reactive oxygen species accumulation seems to be both early and strong, and some genes involved in cell-wall reinforcement are up-regulated.

16h45-17h00: Root endophytes in vegetable crops: Can *Piriformospora indica* support tomato mineral nutrition?

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Plant mutual associations range from pathogenic to mutually beneficial and among others, such interactions depend on the nutritional status of the host. Root colonizers such as arbuscular mycorrhizal fungi (AMF) support plant mineral nutrition in exchange for photosynthetic carbon. The fungus *Piriformospora indica* belonging to the order Sebacinales (Basidiomycota) is a root endophyte with a broad host spectrum. In contrast to AMF, *P. indica* is able to colonize roots and promotes plant growth independent of phosphate concentrations in the soil. Its impact on mineral nutrition is currently under debate. Improved phosphate or nitrogen uptake could not be shown in barley plants, but diminished phosphate amounts in maize plants were detected when they were colonized by a phosphate transporter knocked out *P. indica* mutant. In order to get more insights into the relationship between *P. indica* and plants concerning mineral nutrition *in vitro* and *in vivo* experiments including compartment systems were carried out. Results of these experiments will be presented and discussed.

17h00-17h15: QualiRedFruits: A European effort to develop new agricultural practices for quality production for red fruits enriched in healthy compounds*

Tommaso Sozzi[6], Gianinazzi N[1], Molan P[2], Gollotte A[3], Tiradani L[4], Dieffenbach R[5], Gianinazzi S[6], Gianinazzi-Pearson V[6], Walters D[7], Carlen C[8], Camps Z[8], Susek A[9], Batchvarova R[10], Kondakova V[10], Massardier P[11], Brosse C[11], Loisy J-L[12]
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The QualiRedFruits project is co-funded by the European Commission under the 7th Framework Programme. It brings together 7 SMEs and 5 universities and research centers, and deals with the competitiveness of raspberry production and market. The aims of the project are to create a new market of raspberry with higher quality and to improve the already existing one. The strategy is to develop innovative cultural practices respectful of the environment – natural plant defense elicitor treatment and biotisation with beneficial microorganisms including arbuscular mycorrhizal fungi

(AMF) – and identifying raspberry varieties with higher AOM content. Molecular markers are being developed for the identification of beneficial microorganisms and for the assessment of plant sanitary status. A cryo-preservation protocol is being developed for germplasm long-term conservation of varieties with higher quality. These approaches are being tested from *in vitro* culture to the field. Finally, recommendations for quality production of raspberry to SMEs will be drawn.
* This summary reflects only the authors' views. The EC is not liable for any use that may be made of the information contained therein

17h15-17h30: Advances in *in vitro* culture of AM fungi

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In vitro inoculum production of arbuscular mycorrhizal (AM) fungi has several advantages over traditional systems. In particular, it allows an important production of contaminant-free fungal propagules in a short time, and it is more economical. Therefore, our goal is to develop *in vitro* inoculum production of AM fungi of interest. This goal has been achieved with a *Glomus* sp. originating from Symbiom, Czech Republic. Until now, the molecular characterization on mitochondrial and nuclear DNA has not permitted to distinguish this isolate from *Glomus irregulare* (Syn. *Rhizophagus irregularis*; DAOM197198). However, the *Glomus* sp. isolate presents morphological (white drop-shape spores) and physiological (faster growth) characteristics different from those of DAOM197198, and new molecular approach should be developed to distinguish it.

17h30-17h45: Endophytes in Biotechnology and Agriculture – New COST Action FA1103

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The new COST-Action "Endophytes in Biotechnology and Agriculture" combines activities of different European laboratories in a multi- and interdisciplinary approach to compare protocols and technologies for the analysis and utilization of bacterial and fungal endophytes, mainly for plant production, but also as sources for other applications.
Plants are associated with micro– and nanoorganisms: Endophytic bacteria and fungi, which live inter- and intracellularly in plants without inducing pathogenic symptoms, interact with the host biochemically and genetically. Endophytic microorganisms (EMOs) may function as plant growth and defense promoters by synthesising phytohormones, producing biosurfactants, enzymes or precursors for secondary plant metabolites, fixing atmospheric nitrogen and CO_2 or control plant diseases as well as providing a source for new bioactive natural products with utility in pharmaceutical, agrochemical and other LifeScience applications. The use of these EMOs to control plant-pathogenic bacteria and fungi is receiving increasing attention as a sustainable alternative to synthetic pesticides and antibiotics. Furthermore, these EMOs are likely to be adapted to the presence and metabolism of complex organic molecules and therefore show useful biodegradation activities. In order to reduce the input of pesticides and fertilizers and to bring European added value to an eco-friendly agriculture, it will be important to develop inocula of

biofertilizers, stress protection and biocontrol agents. The aim of the action is to identify bottlenecks limiting the use of endophytes in biotechnology and agriculture and to provide solutions for the economically and ecologically compatible exploitation of endophytes.

17h45-18h00: Mycorrhizal biotechnology: an opportunity for young Masters to contribute to the development of ecofriendly plant production systems

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In developing eco-friendly agriculture, special attention is given to ecosystem services provided to society by beneficial microorganisms (e.g. rhizobia, mycorrhizal fungi, endophytic microorganisms …). Arbuscular mycorrhiza, ancestral symbioses between plant roots and beneficial soil fungi (Glomeromycota), are major actors of these ecosystem services. This association is well known for its beneficial impacts on plants through increased access to nutrients and water, higher resistance against biotic and abiotic stresses, tolerance of contaminated soils, and increased quality of plants and their products (Gianinazzi et al. 2010). However, the development of agricultural chemicals has profoundly modified plant production systems and their management. The massive use of chemical inputs on crops has been done to the detriment of the biological and beneficial life of soils, in particular of mycorrhizal fungi. Soil biofertility decreases, fungal biodiversity is reduced, agricultural systems run out of steam…
Therefore, the development of an industry providing mycorrhizal inocula and services for their management in plant production systems represents an opportunity for young Masters to contribute to the establishment of biological alternatives for promoting sustainable agriculture. In my talk, I will discuss this in relation to my experience in the creation of the company INOCULUMplus.