

January 18, 2019

# Zürich Mycology Symposium 2019

ETH Zürich, ML E 12, 8092 Zürich

08:30 - 09:00 *Arrival and Registration* (fee CHF 30.- in cash only)

## ***Morning session I: Host-parasite interactions*** (chaired by *Andrea Sanchez-Vallet*)

09:00 - 09:05 *Opening remarks* (*Andrea Sanchez-Vallet*)

09:05 - 09:25 **Alejandro Gimeno, Agroscope, Reckenholz**  
Effective biological control of *Fusarium graminearum* from the laboratory to the field using the mycoparasite fungus *Clonostachys rosea*

09:25 - 09:45 **Alessio Bernasconi, Plant Pathology, ETH Zürich**  
How mixed infections affect transmission of the wheat pathogen *Z. tritici*

09:45 - 10:05 **Dr. Francesca Dennert, Institute of Forest, Snow and Landscape Research WSL, Birmensdorf**  
Inoculation experiments: a useful tool for detecting potentially harmful fungal pathogens of trees

10:05 - 10:25 **Dr. Claire E. Stanley, Agroscope, Reckenholz**  
Fungi-on-a-Chip: Unravelling microbe-microbe interactions using microfluidic technology

10:25 - 10:55 *Coffee break*

## ***Morning session II: Molecular / cellular Biology*** (chaired by *Andrea Sanchez-Vallet*)

10:55 - 11:15 **Carolina Francisco Sardinha, Plant Pathology Group, ETH Zurich**  
Phenotypic plasticity of *Zymoseptoria tritici* and its importance for pathogen fitness

11:15 - 11:35 **Prof. Clara Sánchez-Rodríguez, Plant Cell Biology, ETH Zürich**  
The cell biology of root colonization by vascular fungi

11:35 – 12:25 **Microsynth Lecture: Fungus Wars**  
**Prof. Lynne Boddy, Cardiff School of Biosciences, UK**

12:25 – 13:40    **Lunch break (on your own)**

***Afternoon session I: Diversity (chaired by Alexey Mikaberidze)***

- 13:40 - 14:00    **Iva Franić, CABI (WSL), Délémont**  
Fungal diversity in tree seeds from trade and botanic gardens
- 14:00 - 14:20    **Dr. Lucrezia Pardi-Comensoli, EMPA Dübendorf**  
Investigating the potential of microbes for the degradation of epoxy resin related materials
- 14:20 - 14:40    **Dr. Saskia Bindschedler, Laboratory of Microbiology, University of Neuchâtel**  
Harnessing bacterial-fungal interactions for metal biorecovery and bioremediation in urban waste streams
- 14:40 - 15:00    **Dr. Aaron Fox, Agroscope Reckenholz**  
Agricultural management and agro-climatic region are important determinants of fungal community composition in European permanent grasslands
- 15:00 - 15:30    **Coffee break**

***Afternoon session II: Genomics/transcriptomics (chaired by Alexey Mikaberidze)***

- 15:30 - 15:50    **Dr. Florian Freimoser, Agroscope, Wädenswil**  
Snf2 controls pulcherriminic acid biosynthesis and connects pigmentation and antifungal activity of the yeast *Metschnikowia pulcherrima*
- 15:50 - 16:10    **Ziming Zhong, Plant Pathology Group, ETH Zürich**  
QTL mapping reveals the genetic architecture of oxidative stress tolerance in *Zymoseptoria tritici*
- 16:10 - 16:30    **Simang Champramary, Institute of Forest, Snow and Landscape Research WSL, Birmensdorf**  
Rhizomorph formation in *Armillaria ostoyae*: the contribution of rhizomorph-specific genes
- 16:30 - 16:35    **Closing remarks (Markus Künzler)**

Organization: Bruce McDonald, Markus Künzler and Janine Gremion

## Abstracts - Zürich Mycology Symposium, January 18<sup>th</sup> 2019, ETH Zürich

Alejandro Gimeno Agroscope, Reckenholz

### **Effective biological control of *Fusarium graminearum* from the laboratory to the field using the mycoparasite fungus *Clonostachys rosea***

The orientation towards sustainable agricultural systems requires innovative and integrated methods for the control of *Fusarium Head Blight* in wheat to reduce the risk of mycotoxins that contaminate food and feed. In consequence, preventive actions against the dominating causing pathogen species *Fusarium graminearum* (teleomorph *Gibberella zeae*) using microbial biological control agents (BCA) on pathogen-harboring crop residues is raising increased interest to minimise the application of fungicides during the cropping season. Given the vast amount of candidate BCAs however, efforts need to focus on microbes with a proven tolerance and activity against mycotoxin accumulation and a saprophytic lifestyle that is adapted to the environment. Within the scope of the Horizon 2020 project MycoKey this PhD project thoroughly investigated the ability of the mycoparasitic fungus *Clonostachys rosea* to prevent mycotoxin contamination via the inhibition of *F. graminearum* on maize residues.

At first, a laboratory experiment was conducted to confirm the antagonistic activity of *C. rosea* on maize stalk pieces infected with *F. graminearum*, either 48 hours before, simultaneously or 48 hours after the treatment. The results show that in contrast to other fungal antagonists tested, only *C. rosea* strain 016 significantly inhibited the formation of perithecia as well as the discharge of ascospores on previously infected residues. Following this finding, a field experiment was initiated in autumn 2016 and repeated in 2017 to study the antagonistic effect under field conditions and to compare between different formulations of *C. rosea* strain 016. The data collected from the field includes the monitoring of *Fusarium* spp. spore dispersal during the infection period in late spring, the disease rating, the yield parameters, quantification of the mycotoxins deoxynivalenol (DON) and zearalenone (ZEA), as well as the quantification of pathogen DNA in the harvested grain. The latest results will be presented and discussed.

Alessio Bernasoni Plant Pathology, ETH Zürich

### **How mixed infection affects transmission of the wheat pathogen *Z. tritici***

Natural pathosystems frequently involve several strains rather than a single one. Pathogens that share resources and/or face the same immune responses interfere with each other. Co-infecting strains have the potential to affect the outcome of the infection, the epidemiology and transmissibility of the pathogen. However, so far, only few studies have tackled the infection dynamics of mixed infections. This is also the case for septoria leaf blotch, a wheat disease caused by the necrotrophic pathogen *Zymoseptoria tritici*. *In natural conditions, even a single lesion can be produced by multiple strains.* In our work we aim to shed light on the dynamics of within-host interaction between different *Z. tritici* strains. Our recent study has shown that the outcome of mixed infections could not be predicted based on analyzing infections produced by single strains. We have shown that competition between strains of *Z. tritici* leads to changes in the overall virulence and to a reduction in the expected number of pycnidia formed. In transmission experiments using mixed infections, we demonstrated that one strain was able to outcompete the co-infecting others. We observed that either prudent or highly virulent strains were selected depending on the cultivar used, indicating that the evolution of virulence in mixed infections of *Z. tritici* is highly dependent on the host and not on the virulence phenotype of the strain. We suggest that high adaptation of each strain to a specific cultivar confers increased competition capacity and provides an advantage to the strain to be successfully transmitted in co-infections. Thus, our data highlights that within-host competition impacts on the virulence outcome and on the evolution of virulence of septoria tritici blotch. Deepening our understanding of mixed infection dynamics is required to develop novel control strategies against this damaging pathogen.

Dr. Francesca Dennert, Institute of Forest, Snow and Landscape Research WSL, Birmensdorf

### **Inoculation experiments: a useful tool for detecting potentially harmful fungal pathogens of trees**

Invasions by non-native pests and pathogens represent a major threat to natural ecosystems worldwide. The best way to reduce the impact of pest invasions is to identify possible invaders before they leave their country or origin and then prevent their introduction into new countries. Here, we show the utility of *in vitro* inoculation experiments for determining pathogenicity and host range of invasive fungal pathogens, on the example of the chestnut blight fungus *Cryphonectria parasitica* and related *Cryphonectria* species.

In the first experiment, we aimed at determining the pathogenicity of three *Cryphonectria* species towards potential hosts in the family Fagaceae. For this, seedlings of European chestnut (*Castanea sativa*), pedunculate oak (*Quercus robur*) and European beech (*Fagus sylvatica*) were inoculated in the WSL biosafety greenhouse with three genotypes of each *C. parasitica* (Asian species, invasive to Europe), *C. naterciae* (European species), and *C. japonica* (Asian species, not present in Europe). The size of the developing lesion was measured and mortality assessed every other week for four months. The highest damage was caused by *C. parasitica* on European chestnut, with no significant differences among genotypes. *C. japonica* and *C. naterciae* induced significantly smaller lesions on this host species. In general, pedunculate oak was more susceptible to all three *Cryphonectria* species than European beech.

In the second experiment, we investigated the potential consequences that an accidental introduction of extra-European *C. parasitica* genotypes (intraspecific invasion) could have for the chestnut blight epidemics in Europe. For this, *C. parasitica* genotypes from South Korea, North America, and Europe were inoculated on seedlings of European chestnut using the same method as for the first experiment. Four months after inoculations, only 9 out of the 155 inoculated seedlings were still alive. Detailed genotype-based analyses are still ongoing and their results and significance will be discussed in the presentation.

Dr. Claire E. Stanley, Agroscope, Reckenholz

#### **Fungi-on-a-Chip: Unravelling microbe-microbe interactions using microfluidic technology**

Soil is one of the most complex systems on Earth, governed by numerous physical, geochemical and biological processes, and provides the ecosystem services vital for terrestrial life. This 'material' supports a myriad of plants, microorganisms and microfauna and hosts a complex array of interactions taking place between these living elements. However, despite the importance of microbes in soil functioning, there exists a major knowledge gap concerning the function and dynamics of the soil microbiome and influence of the physio-chemical environment upon microbial interaction and communication at the cellular level.

Recently, it has been demonstrated that microfluidic technology can offer new opportunities to study whole living organisms and their interactions and has a great potential to provide a unique view of biological events at the level of single organisms and cells (i.e. microbe-microbe interactions). I have developed microfluidic systems to probe interactions between fungi, bacteria and nematodes, which has revealed novel insights into the antagonistic strategies of these microorganisms including bacteria-induced blebbing of hyphal cells, as well as discovering that undifferentiated mycelium can communicate within certain microdomains using previously unknown specialised hyphae. We are now developing new microfluidic tools to investigate microbial interactions in the rhizosphere, specifically to probe the cell biology and physiology of microbe association and colonisation of arbuscular mycorrhizal fungi at the cellular level.

Carolina Sardinha Francisco, Plant Pathology Group, ETH Zürich

#### **Phenotypic plasticity of *Zymoseptoria tritici* and its importance for pathogen fitness**

During their life cycles, pathogens have to adapt to many biotic and abiotic environmental stresses to maximize their overall fitness. Morphological transitions are one of the least understood of the many strategies employed by fungal plant pathogens to adapt to constantly changing environments, even though different morphotypes may play important biological roles. We characterized the responses of the wheat pathogen *Zymoseptoria tritici* to a series of environmental stresses in order to understand the effects of changing environments on fungal morphology and adaptation. We found that all tested stresses induced morphological changes, but different responses were found among four different strains. A transcription analysis showed that morphogenesis and virulence factors are co-regulated. We discovered that *Z. tritici* forms chlamydospores and demonstrated that these structures are better able to survive extreme cold, heat and drought than other cell types. We also show that blastospores (the "yeast-like" form of the pathogen typically found only in laboratory conditions) can form from germinated pycnidiospores on the surface of wheat leaves, suggesting that this morphotype can play an important role in the natural history of *Z. tritici*. Our findings illustrate how changing environmental conditions can affect cellular morphology and lead to the formation of new morphotypes, with each morphotype having a potential impact on both pathogen survival and disease epidemiology.

Prof. Clara Sánchez-Rodríguez, Plant Cell Biology, ETH Zürich

#### **The cell biology of root colonization by vascular fungi**

Most vascular wilts are caused by ubiquitous soil-borne bacteria, fungi and oomycetes that invade the root host intercellularly until reaching the xylem vessels, where they proliferate. Considering the inefficiency of biological, chemical and cultural methods to control plant vascular diseases, and their impact on natural ecosystems (ecologic) and food and feed production (economic), an exhaustive molecular study of the interaction is crucial. One of our main objectives is delivering valuable information about the interaction during the first stage of plant colonization, when the microbe grows intercellular. This initial infection phase requires a strong host cell wall alteration and a concomitant response and growth adaptation from both organisms. With that purpose, we follow a multidisciplinary approach employing molecular and in vivo high definition microscopy at cellular and temporal resolution, using the model pathosystem *Arabidopsis thaliana*-*Fusarium oxysporum*.

Iva Franić, CABI (WSL), Délémont

#### **Fungal diversity in tree seeds from trade and botanic gardens**

Trade in plants for planting is the main pathway of introduction for alien fungal pathogens (AFP) of forest trees. Although belonging to this pathway, seeds are considered to be safer than other plants for planting and their trade is, thus, less regulated. Seed borne fungi are, however, generally not well known because previous studies have focused on commercially important tree species. We assessed the fungal diversity of Angiosperm and Gymnosperm tree seeds obtained from commercial seed suppliers from the native area of tree species from three continents (Europe, North America and Asia). The seed borne fungal community was characterized by high-throughput sequencing and, for a subset of samples, by plating on agar. Results show that these communities are highly diverse, with Angiosperm seeds and seeds from Asia having a higher diversity and a different fungal community composition than Gymnosperm seeds and seeds from the other two continents. Currently, we are analysing a larger data set, including also seeds from arboreta and the exotic range of the tree species, for testing three ecological hypotheses about patterns of fungal diversity: (1) the diversity is lower on exotic than native tree species, (2) the difference in fungal species community composition increases and the diversity decreases with increasing distance from the native range, and (3) the fraction of fungal species shared between native and exotic tree species is negatively related to the phylogenetic distance between the tree species. The assessed patterns of fungal diversity will be presented and discussed from the phytosanitary perspective.

Dr. Lucrezia Pardi-Comensoli, EMPA Dübendorf

#### **Investigating the potential of microbes for the degradation of epoxy resin related materials**

Epoxy resin is a synthetic thermoset produced by the reaction of a bisphenolic compound with an amine or hydroxide based hardener. This extremely resistant polymer is increasingly used in many engineering

applications, as coating, for protecting metal from corrosion, or as matrix for carbon fiber reinforced materials (CFRP). Due to its high stability against thermal and chemical degradation its recycling is extremely difficult. Therefore an efficient and sustainable methodology for the disposal and/or recycling of this material is still not available. Different strategies are currently under consideration for example for the recycling of CFRP composites, but these techniques require large amounts of energy, employ aggressive chemicals, or produce an undesirable emission of toxic gases.

During evolution microbes have adapted to a range of severe environments developing countless metabolic abilities that enable growth. In this project their potential for the biodegradation of epoxy related materials is being explored. An overview of the preliminary results will be presented here.

Firstly, a screening of potential microbes to degrade epoxy resins was performed with selected fungal strains. Secondly, mass loss quantification, colorimetric, and UV-Visible investigations were carried out on epoxy samples incubated in soil microcosms for 1, 3 and 6 months. On the same samples changes in selected mechanical properties (hardness and flexure) and of surface chemistry (Fourier transform infrared spectroscopy and contact angle) were evaluated. Finally studies of microbial colonization of epoxy samples exposed to water were also performed with environmental scanning electron microscopy.

Dr. Saskia Bindschedler Laboratory of Microbiology, University of Neuchâtel

#### **Harnessing bacterial-fungal interactions for metal biorecovery and bioremediation in urban waste streams**

Urban waste is a growing fraction of the waste produced worldwide. It consists of both inorganic (e.g. discarded electronic equipment - e-waste), and organic waste (e.g. digested sewage sludge). Both contain valuable compounds such as precious metals and phosphorous, which are non-renewable resources. To sustain the development of modern societies, the demand for these resources will keep on growing, along with the depletion of natural mining sites. This results in environmental, ethical, and economical concerns. This has led to the development of the concept of urban-mining, which proposes to use urban and industrial waste as a mine for traditionally non-renewable resources. At present-day, the recycling of industrial waste is still in its infancy. Current methods in metal recycling consist in relatively polluting approaches (pyro- and hydrometallurgy). As a result, industrial waste recycling is a timely issue that requires innovative and sustainable approaches. Industrial waste typically consists of a heterogeneous matrix of materials and thus a parallel can be drawn to other complex systems such as soils. Microbial interactions in soil are essential to maintain biogeochemical cycles. In this project, we aim at exploring the possibility of using bacterial-fungal interactions (BFI) to develop an innovative process for the biorecovery of selected valuable compounds from industrial waste. We propose to harness both, BFI and the fungal highway (FH) mechanism as a logistic tool. The idea is to take advantage of bacterial and fungal biogeochemical capabilities towards metals, together with synergistic mechanisms that take place upon BFI and FH in order to recover metals in minute concentration from heterogeneous matrices such as e-waste and digested sewage sludge. The experimental design consists in enrichment and isolation of metal-resistant bacterial-fungal consortia from metal-rich samples, followed by the assessment of their metal mobilization and immobilization capabilities. In parallel, microcosm trials with actual industrial waste are set-up to design a process that could be scaled-up. By harnessing the natural interactions that exist between microbes, along with their ability to act as chemical reactors, an ecological, economical, and ethical strategy for the biorecovery and/or bioremediation of metals could be developed for the field of urban-mining.

Dr. Aaron Fox, Agroscope Reckenholz

#### **Agricultural management and agro-climatic region are important determinants of fungal community composition in European permanent grasslands**

The BIOINVENT project aims to further our understanding of how the composition of the grassland microbiome is influenced along gradients of both management and agro-climatic regions in Europe. For a pan-European survey, a North-South transect was established with 5 participating countries: comprising Sweden, Germany, Switzerland, Portugal (mainland) and the Azores. Each country was divided into a 'favorable' agricultural region, where productivity is optimal and a 'less favorable' region where productivity may be constrained by certain environmental/climatic conditions. In the summer of 2017, soil samples from 3 permanent grasslands management types; intensive, low intensive and extensive, were taken from all regions (288 sites in total). The fungal internal transcribed spacer region (ITS) was PCR amplified from these samples and an amplicon-based Illumina Miseq sequence analysis was conducted. Agro-climatic region (country plus favourability region) had a highly significant influence on fungal community composition ( $P < 0.001$ ). Furthermore, the fungal community significantly differentiated according to favourability region in each country with the exception of the Azores. Management type also displayed a significant effect, with the intensive management harbouring a significantly distinct fungal community when compared to both the low intensive and extensive managements ( $P < 0.05$ ). Agro-climatic region was, however, the stronger driver of fungal community composition compared to management along the transect. Further work will examine bacterial community composition in the same samples. The knowledge gained on how both agro-climatic region and management influence the grassland microbiome will be used to develop strategies for monitoring soil microbial biodiversity trends in these systems.

Dr. Florian M. Freimoser, Agroscope, Wädenswil

#### **Snf2 controls pulcherriminic acid biosynthesis and connects pigmentation and antifungal activity of the yeast *Metschnikowia pulcherrima***

The yeast *Metschnikowia pulcherrima* synthesizes the red pigment pulcherrimin, from cyclodileucine (cyclo(Leu-Leu)) as a precursor, and exhibits strong antifungal activity against notorious plant pathogenic fungi such as

Botrytis and Gibberella (i.e., Fusarium). It therefore has great potential for biocontrol applications against fungal diseases; particularly in the phyllosphere where this yeast is frequently found.

This multidisciplinary study combined functional assays, whole genome and transcriptome sequencing, genetic complementation, and state of the art mass spectrometry to characterize a naturally occurring, pigmentless, weakly antagonistic *M. pulcherrima* mutant. A de novo assembled reference genome of the wildtype served as the basis for identifying a point mutation that causes a premature stop codon in the transcriptional regulator SNF2 in the mutant strain. Complementation of the *snf2* mutant strain with the wildtype SNF2 gene restored pigmentation and recovered the strong antifungal activity of *M. pulcherrima* against plant pathogens in vitro and on cherries. Ultra-performance liquid chromatography-high resolution heated electrospray ionization mass spectrometry (UPLC HR HESI-MS) analyses confirmed the presence of cyclo(Leu-Leu) and pulcherriminic acid in *M. pulcherrima* culture supernatants, but also identified a novel precursor and potential degradation products. All of these compounds were identified in the wildtype and complemented strain, but were undetectable in the pigmentless *snf2* mutant strain. The nature of these degradation products implies an enzymatically controlled catabolism of pulcherriminic acid and/or pulcherrimin, which opens up new avenues for research on the metabolism and functions of pulcherrimin. These results thus identify SNF2 as a regulator of antifungal activity and pulcherriminic acid biosynthesis in *M. pulcherrima* and provides a starting point for deciphering the molecular functions underlying the antagonistic activity of this yeast and the development of biocontrol applications employing *M. pulcherrima*.

Ziming Zhong, Plant Pathology Group, ETH Zürich

#### **QTL mapping reveals the genetic architecture of oxidative stress tolerance in *Zymoseptoria tritici***

Reactive oxidative species (ROS) are involved as signals to induce host defense and as weapons to isolate pathogens during plant-pathogen interactions. Efficiently neutralization of ROS during infection have been shown to favor pathogen invasion. However, due to the considerable plasticity and complexity of the oxidative response system, the genes involved in this response and what roles do they play in planta are not yet clear. In this study, we performed quantitative trait locus mapping in *Z. tritici*, a devastating wheat pathogen in Europe, in order to investigate the genetic architecture of oxidative stress tolerance. Fifteen significant QTLs were found for the growth under 1 mM hydrogen peroxide in two Swiss cross populations. One catalase (*ZtCat1*), two catalase-peroxidases (*ZtCpx1* and *ZtCpx2*), one glutathione synthetase, and two glutathione S-transferases were found within the significant QTLs, and they may be associated to the variation in oxidative stress tolerance among strains. In planta expression of these candidate genes and their high homology to known catalases and peroxidases from other fungi suggest that these genes may play important roles in eliminating exogenous ROS during in planta infection. Furthermore, our results suggests that one of the catalase-peroxidase (*Cpx1*) in *Z. tritici* is only expressed at high dose of exogenous oxidative stress, and previous study showed that this gene does not affect virulence. Our study not only confirms the importance of cooperation of catalases, peroxidases and the glutathione system in in planta infection, but it also suggests two stages of exogenous oxidative burst and one stage of endogenous oxidative stress during infection in planta. Our results also sheds light on the possible roles of ROS-related genes in the aggressiveness and quantitative virulence of phytopathogens.

Simang Champramary, Institute of Forest, Snow and Landscape Research WSL, Birmensdorf

#### **Rhizomorph formation in *Armillaria ostoyae*: the contribution of rhizomorph specific genes**

*Armillaria* (Basidiomycota, Agaricomycetes) species are primary decay drivers in forest ecosystem processes and amongst the most prominent killers, decayers of conifers and hardwoods. They are easily recognised by their rhizomorphs, strand-like fungal organs that, similarly to that of plant roots, spread in the soil foraging for food and an inhabitable environment. Rhizomorphs form large underground fungal networks and can also persist under unfavorable conditions. Most importantly, rhizomorphs act as the exploratory organs to contact plant roots where they obtain nutrients by penetrating the roots, and they are also well-suited for solute diffusion and nutrient transport.

So far, more than 10 genome-sequencing projects of *Armillaria* and *Desarmillaria* genomes have been completed, and six genomes have already been published to date (1). Recent transcriptomic analyses revealed developmental similarities between rhizomorphs and fruiting bodies confirming shared multicellular origin for functionally different structures (2).

Our current interest is to understand, what makes rhizomorphs different from the vegetative mycelia and fruiting bodies. Which genes might be responsible in helping the formation of such specialized structures like the rhizomorphs.

In this study, we used RNA-Seq-based whole genome level differential expression profiles in *Armillaria ostoyae* (C18) to identify genes which might serve as driving factors for the formation of rhizomorphs. 191 rhizomorph specific genes showing log fold change of 2 or above compared to different developmental conditions (vegetative mycelia, primordial stage, fruiting body cap, lamellae and stipe) were identified. We also found 231 genes shared by rhizomorphs and vegetative mycelia. Among the rhizomorph specific genes (191) we noted the significant enrichment for soluble secretory genes and several lineage-specific genes seem to represent the unique features of the rhizomorphs.

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