

January 19<sup>th</sup> 2018

# Zürich Mycology Symposium 2018

Agroscope Reckenholz in Zürich-Affoltern  
Reckenholzstrasse 191 8046 Zürich

**08:30 - 09:00** *Arrival and Registration* (fee CHF 10.-)

## ***Morning session I: Plant pathogens - Life cycle and possible control measures*** (chaired by Jürg Enkerli)

**09:00 - 09:05** *Opening remarks*

**09:05 - 09:25** **Ludwig Beenken (Group Valentin Queloz) Institute of Forest, Snow and Landscape Research WSL, Birmensdorf**  
A new leaf pathogen of *Fagus sylvatica* in Europe

**09:25 - 09:45** **Dimitrios Drakopoulos (Group Vogelgsang) Agroscope, Reckenholz**  
Use of botanicals to combat *Fusarium graminearum* (*Gibberella zeae*): An in vitro approach tackling the entire life cycle

**09:45 - 10:05** **Hansjakob Schärer, FiBL Research Institute for Organic Agriculture, Frick**  
Multi-dimensional approach against *Marssonina coronaria* in apple

**10:05 - 10:25** **Ursula Oggenfuss (Group Croll), Institute of Biology, University of Neuchâtel**  
A population-level invasion by transposable elements in a fungal pathogen

**10:25 - 10:55** *Coffee break*

## ***Morning session II: Antagonists of fungal pathogens / Antifungal activities*** (chaired by Jürg Enkerli)

**10:55 - 11:15** **Isha Jamil (Group Junier), Institute of Biology, University of Neuchâtel**  
Targeted mycophagous lifestyle of *Lysinibacillus sphaericus* against the phytopathogenic fungus *Rhizoctonia solani*

**11:15 - 11:35** **Fanny Louviot (Group Weisskopf, Mauch) Department of Biology, University of Fribourg**  
Isolation, identification and screening of Actinobacteria with antagonistic activity against *Botrytis cinerea*

**11:35 - 11:55** **Denise Müller (Group Freimoser), Agroscope, Wädenswil**  
Identifying the mode of antagonism of the yeast *Metschnikowia pulcherrima* against fungal plant pathogens

**11:55 - 12:15** **Etter Danai (Group Drissner), Agroscope, Wädenswil**  
Inactivation of moulds by low-energy electron beam treatment

12:15 - 13:30 **Lunch break** (in front of the Aula)

13:30 - 14:20 **Microsynth-Lecture**

**Prof. em. Rosmarie Honegger, Department of Plant and Microbial Biology, University of Zurich.**

Fungal diversity 400 Myr ago: Early Devonian fossils from siltstone and Rhynie chert deposits

***Afternoon session I: Animal and plant pathogens - Interactions with the host*** (chaired by Franco Widmer)

14:20 - 14:40 **Florian Sparber (Group LeibundGut-Landmann), Vetsuisse Faculty, University of Zurich**

Type-17 immunity controls Malassezia-mediated skin infection

14:40 - 15:00 **Lukas Meile (Group McDonald), Institute of Integrative Biology, ETH Zürich**

Isolate-specific recognition triggers quantitative resistance in wheat

15:00 - 15:30 **Coffee break**

***Afternoon session II: Fungal diversity in different habitats*** (chaired by Franco Widmer)

15:30 - 15:50 **Ivano Brunner, Institute of Forest, Snow and Landscape Research WSL, Birmensdorf**

Jules Favre - a pioneer of alpine mycology

15:50 - 16:10 **Salome Schneider (Group Rigling), Swiss Federal Research Institute WSL, Birmensdorf**

High throughput monitoring of *Dothistroma* and *Lecanosticta* needle blight in Switzerland

16:10 - 16:30 **Banerjee Samiran Agroscope (Group van der Heijden) Agroscope, Zürich**

Impact of land-use intensity on root fungal network complexity and the abundance of keystone taxa

16:30 - 16:35 **Closing remarks**

Organization: Jürg Enkerli and Franco Widmer

## Abstracts - Zürich Mycology Symposium, January 19<sup>th</sup> 2018, Agroscope Reckenholz

Ludwig Beenken (Group Valentin Queloz) Institute of Forest, Snow and Landscape Research WSL, Birmensdorf

### **A new leaf pathogen of *Fagus sylvatica* in Europe**

"The European beech, *Fagus sylvatica*, is the dominant deciduous tree species in Switzerland and over large parts of central Europe. Up to now, no introduced alien pathogen was known infecting leaves of this important tree species. 2008, a new leaf blotch disease was discovered on beech in Switzerland and, soon after, in Germany. This new disease was provisionally assigned to the beech pathogen *Pseudodidymella fagi* (Dothideomycetes, Ascomycota), first described on *Fagus crenata* in Japan. The asexual "Mycopappus" morph of *Ps. fagi* is formed on necrotic leaf spots of green leaves of beech during summer and autumn. In early spring, its sexual ascospores are formed on beech leaves of the precedent year in the litter of *F. sylvatica* and are assumed to infect the newly formed leaves of beech trees.

In the present study, we aim to answer the following questions: How serious is the damage of beech trees caused by *Ps. fagi*? Is *Ps. fagi* really an introduced species or a yet undetected indigenous species in Europe? In an infection experiment, we could show that *Ps. fagi* harm shade leaves of beech faster and stronger than sun-exposed leaves. Half of the leaves were perforated to imitate the influence of insect feeding on the infection rate. Whereas shade leaves did not show differences between both treatments, the perforated sun leaves were infected faster. To estimate the genetic diversity within the European populations of *Ps. fagi*, we use ISSR-PCR. The genetic diversity of *Ps. fagi* is quite low in Europe that would be expected of recently introduced organisms.

Lit.: Gross A, Beenken L, Dubach V, Queloz V, Tanaka K, Hashimoto A, Holdenrieder O (2017) *Pseudodidymella fagi* and *Petrakia deviata*: Two closely related tree pathogens new to central Europe. For Path. 2017;00:e12351. <https://doi.org/10.1111/efp.12351>"

Dimitrios Drakopoulos (Group Vogelgsang) Agroscope, Zürich

### **Use of botanicals to combat *Fusarium graminearum* (*Gibberella zeae*): An in vitro approach tackling the entire life cycle**

Fusarium Head Blight (FHB) is one of the most important cereal diseases worldwide causing not only significant reductions in grain yield but also severe contaminations of the harvested products with mycotoxins that jeopardise food and feed safety. The predominant species of the FHB disease complex is *Fusarium graminearum* (FG; teleomorph *Gibberella zeae*). Within the context of the Swiss national action plan to minimise the risk of plant protection products and to improve food and feed safety, the main objective of this study is to assess the control efficacy of different natural substances (i.e., botanicals) on essential parts of the life cycle of three FG strains. The botanicals included two mustard extracts (Pure Yellow Mustard (PYM) and Tillecur (Ti)) and suspensions of milled Chinese galls (CG) in water. PYM and Ti greatly inhibited the germination of conidia (anamorph) and ascospores (teleomorph) at 1% and 2% concentrations, while CG was only effective against conidia germination at the same concentrations. The discharge of ascospores was 2- to 4-fold lower when botanicals were applied on mature perithecia, the fruiting bodies of *G. zeae*. Mycelium growth was suppressed with the use of CG at 0.5% and 1%, PYM at 4%, while Ti showed no influence. To better mimic field conditions, the effect of botanicals on perithecia formation using FG-inoculated maize stalks is currently being tested. The results of the present study demonstrate a great potential to control FG with non-conventional crop protection measures using botanicals with antifungal properties.

Hansjakob Schärer, FiBL Research Institute for Organic Agriculture, Frick

### **Multi-dimensional approach against *Marssonina coronaria* in apple**

*Marssonina* leaf blotch is a fungal disease of apple caused by the ascomycete *Diplocarpon mali* (anamorph: *Marssonina coronaria*). The pathogen originates from Asia where it accounts for crop losses of up to 100%. In Europe it was first detected in Italy in 2001 and is spreading since then in Switzerland and southern Germany (first reports in 2010), mainly in organic apple orchards and in orchards for cider production with a reduced fungicide regime. *M. coronaria* infects the leaves by using appressoria and shortly afterwards forms haustoria in the host cells. Blackish-violet spots appear on the upper leaf side, sometimes turning into star-like lesions. On the lesions acervuli are produced and eventually the leaves become chlorotic and fall off. First symptoms can be observed in June after a humid period, leaf drop may start two to three weeks later. Severely infected trees can be completely defoliated already in August. Three projects in Switzerland give attention to *Marssonina*: The FiBL and KOB Interreg project aims at gaining more knowledge on the biology of the fungus and on control methods in organic farming. For example has the teleomorph *Diplocarpon mali* not yet been detected in Europe and also the form of overwintering is still unknown. Identification of resistant or less susceptible cultivars, to be directly used in production or as sources of resistance in apple breeding, is a further important pillar in *Marssonina* control which is addressed both in the Interreg project and in the Agroscope projects. The Agroscope project HERAKLES Plus also comprises chemical control strategies. A population study executed in a joint FiBL, Agroscope and ETH Zurich project will provide information on the genetic diversity of the pathogen and may show if the pathogen sexually reproduces in Europe. First results of the research projects will be presented.

Ursula Oggenfuss (Group Croll), Institute of Biology, University of Neuchâtel

### **A population-level invasion by transposable elements in a fungal pathogen**

"Transposable elements (TEs) are important drivers of genome evolution and can impact the expression of phenotypic traits. TEs spread by insertion into novel genomic locations. In the fungal pathogen *Zymoseptoria tritici*, the insertion of TEs contributed to the evolution of fungicide resistance. TEs also triggered chromosomal rearrangements and epigenetic silencing that enabled the pathogen to surmount host immunity. These individual impacts of TEs on phenotypic traits point to a key role of TEs in adaptive evolution. However, the population-level context of TE dynamics is poorly understood. To address this, we analysed whole genome sequences of 245 fungal strains across five populations of *Z. tritici*. We used mapped reads to identify genome-wide TE insertion and deletion sites. A total of 2'161 sites in the genome that were polymorphic for the presence or absence of a TE within the species was identified. A significant excess in rare insertions compared to neutral polymorphism was found, showing that TE insertions were overall under strong negative selection. We focused on a pair of populations that was collected in the same field at an interval of 25 years. Neutral markers showed that the populations diverged only very little by genetic drift, but a small subset of TE loci swept to near complete fixation. These rapid frequency changes driven by strong positive selection point to adaptive roles of individual TE insertions. Our study shows that TE invasion dynamics at the population scale could serve as a model to recapitulate TE-driven genome evolution over deeper evolutionary timescales.

Isha Jamil (Group Junier), Institute of Biology, University of Neuchatel

#### **Targeted mycophagous lifestyle of *Lysinibacillus sphaericus* against the phytopathogenic fungus *Rhizoctonia solani***

Bacteria are well known for their diversified nutritional capabilities, which may lead to positive or negative interactions with other organisms. Mycophagy is a behavior allowing bacteria to obtain nutrients from living fungi, with a negative impact on the fungal partner. Soil is a major hub of bacterial-fungal interactions. Bacilli are an important component of the soil microbial community and several species are widely used in biofertilization and biocontrol. We observed a mycophagous lifestyle in *Lysinibacillus sphaericus* against the soil-borne generalist phytopathogenic fungus *Rhizoctonia solani*. In order to understand the mechanisms underlying this mycophagous lifestyle, a confrontation assay was performed, where *R. solani* biomass and its exudates were the sole carbon source for the bacterium. In order to assess the specificity of the interaction, we also confronted *L. sphaericus* to a common saprophytic soil fungus, *Trichoderma reesei*. *L. sphaericus* inhibited the growth of *R. solani* but not of *T. reesei* and was able to disperse on the fungal hyphae of both fungal species ("fungal highway" dispersal). *R. solani* hyphae that were in direct contact with bacteria were adversely damaged and unable to re-grow and this was not observed for *T. reesei*. Microscopic observations revealed the deformation of *R. solani*'s cell wall and bacteria penetrating fungal hyphae. Bacterial growth occurred at the expense of *R. solani* fungal mycelium only, but no growth was measured on either *T. reesei* hyphal biomass or in fungal exudates of both species. This confirms that the living mycelium of *R. solani* is required for bacterial growth and demonstrates the ability of the bacterium to select its target. This yet unexplored mycophagous lifestyle of *L. sphaericus* could be exploited for the control of phytopathogens in sustainable agriculture.

Fanny Louviot (Group Weisskopf, Mauch) Department of Biology, University of Fribourg

#### **Isolation, identification and screening of Actinobacteria with antagonistic activity against *Botrytis cinerea***

*Botrytis cinerea* is a necrotrophic plant pathogen able to attack more than 200 different host species worldwide, including crop plants, causing strong yield losses. Many synthetic fungicides have been developed to control this pathogen and nowadays agriculture relies heavily on these. Unfortunately, these products have harmful side effects on ecosystem and lead to increased pathogens' resistance to antibiotics. There is therefore an urgent and critical need for bio-based agriculture. There is a large body of literature describing potential uses of bacteria as agents managing plant health. The aim of my master project is to identify Actinobacteria strains showing an antagonistic activity against *Botrytis cinerea* to contribute to solve this pathogen problem in an environmental-friendly way. Actinobacteria are good candidates because they are well-known for their secondary metabolites conferring antimicrobial and/or antifungal activities. Indeed, they are recognized as the most prolific group in antibiotic production. Thus, a large portion of marketed antibiotics originates from Actinobacteria. Because of these features, using them as biocontrol agents could be an efficient mean to deleterious side effects of synthetic fungicides. We have isolated 28 Actinobacteria from different soils and selected the isolates showing an antagonistic activity against *Botrytis cinerea*. Three Actinobacteria isolates were then selected, all belonging to the Streptomyces genus. First results indicate that different stages of *Botrytis cinerea*'s development are affected by the Actinobacteria. Indeed, the cell-free filtrates of these Actinobacteria are able to delay the growth, the germination and the development of *Botrytis cinerea* in vitro. When testing this antagonistic activity on the model plant *Arabidopsis thaliana*, we observed that plants were less sensitive to the necrotrophic attacks once treated with the cell-free filtrates of Actinobacteria. We are currently identifying the compound(s) responsible for this activity against *Botrytis cinerea*.

Denise Müller (Group Freimoser), Agroscope, Wädenswil

#### **Identifying the mode of antagonism of the yeast *Metschnikowia pulcherrima* against fungal plant pathogens**

Fruit and vegetable production is threatened by a plethora of different fungal plant pathogens. To protect crop plants and fulfil the consumer and retailer quality requirements, regular applications of plant protection agents are necessary. However, authorities demand a substantial reduction of health and environmental risks caused by plant protection agents (i.e., at least 50% in Switzerland). To achieve this goal and maintain the production of high-quality fruits and vegetables, novel plant protection strategies need to be established. Therefore, we are assessing the potential of naturally occurring yeasts as antagonists of fungal plant pathogens.

Numerous studies demonstrate the potential of naturally occurring yeasts as plant protection agents, but only few yeast-based products are commercially available and the activity of biocontrol yeasts in the field is often variable. The main reason for this inconsistency is the lack of knowledge about the modes of action required for reliable plant protection in the field. It is thus the goal of our work to identify strongly antagonistic yeasts and to elucidate their modes of action in order to contribute to the long-term goal of developing safe and effective plant protection strategies. A screen of 40 yeast isolates from agricultural samples against 16 filamentous fungi (plant pathogens, saprophytes and soil fungi) identified an isolate of *Metschnikowia pulcherrima* as the overall most antagonistic yeast. To characterise the factors underlying the antagonistic activity of *M. pulcherrima* in detail, we compare the wildtype isolated with a naturally occurring mutant with reduced antagonistic activity. The characterisation of *M. pulcherrima* includes full genome and transcriptome sequencing, quantification of specific factors potentially involved in the mode of antagonism (e.g., 2-phenylethanol), the identification of novel, secreted metabolites.

Etter Danai (Group Drissner), Agroscope, Wädenswil

#### **Inactivation of moulds by low-energy electron beam treatment**

"Low-energy electron beam treatment is a novel non-thermal processing application that can be used to disinfect food surfaces. Electrons are accelerated and can target the sample directly. Microorganisms on the surface are inactivated by DNA-breaks or oxidative damage caused by the electrons. Only the outermost few hundred micrometres are penetrated by the treatment, the foodstuff is therefore barely affected.

In our studies a filter model system was employed to characterise the inactivation kinetics of filamentous fungi and their corresponding spores. Additionally, mould spores were used for the inoculation of food specimens such as raisins followed by an electron beam treatment for sanitation. Complete inactivation of mould spores was achieved on the filter model system. Treatment of mycelia led to morphological changes of fungi. Disinfection of raisins proved that even on challenging three dimensional surface structures electron beam treatment effectively counteracts microbial contaminations.

Florian Sparber (Group LeibundGut-Landmann), Vetsuisse Faculty, University of Zurich

#### **Type-17 immunity controls *Malassezia*-mediated skin infection**

"The constant exposure of our epithelial surfaces to microbes is important for immune homeostasis, but also bears a constant threat to microbial invasion and disease. The skin microbiota comprises not only bacteria but also fungi, of which *Malassezia* spp. are by far the most prominent. There is accumulating evidence that *Malassezia* is involved in the development and/or exacerbation of skin disorders including pityriasis versicolor, seborrhoeic eczema and atopic dermatitis. The causal relationship between the fungus and these pathologies remains however unclear. To gain knowledge about the complex interplay between *Malassezia* and the skin immune system, we established a novel experimental model of *Malassezia* skin infection in mice. This has allowed deciphering the cellular and molecular mechanisms that control the fungal growth on the skin. Topical application of *Malassezia* spp. onto barrier-disrupted murine skin results in a robust immune response to the fungus characterized by skin thickening, infiltration of inflammatory leukocytes and the local production of cytokines and antimicrobial peptides. Infected mice develop a robust Th17 adaptive immune response and induce local production of type 17 cytokines in the skin. Consistent with this, we found that *Malassezia*-specific memory T cells in healthy human individuals that respond to the commensal fungus belong predominantly to the Th17 subset. Finally, we demonstrate that the IL-17 pathway is critical for fungal control because mice deficient in IL-17 production are unable to prevent fungal overgrowth. Together, our results demonstrate a critical role of type 17 immunity in keeping the balance between the skin commensal *Malassezia* and the mammalian immune system. This is reminiscent of what is known about the IL-17 pathway in the control of other fungal commensals such as *Candida* spp. and highlights the relevance of IL-17 in host protection at barrier tissues."

Lukas Meile (Group McDonald), Institute of Integrative Biology, ETH Zürich

#### **Isolate-specific recognition triggers quantitative resistance in wheat**

Adaptation of fungal pathogens to colonize plants often involves escape from host surveillance. This can be mediated by sequence polymorphism of avirulence genes that evolve to evade recognition by host resistance genes. Despite the ubiquity and importance of avirulence genes for the infection outcome, the mechanisms behind their evolution remain unknown. The causal agent of *Septoria tritici* blotch on wheat, *Zymoseptoria tritici*, is a necrotrophic pathogen that is globally distributed. Resistance is mediated by 21 mapped major resistance genes, of which many lead to partial resistance. It still remains largely unknown what components are recognized by these particular resistance genes. In order to elucidate the genetic basis of quantitative virulence, a genetic mapping approach was undertaken. Differences between two isolates were mapped to a transposable element-rich and highly plastic genomic region that included a cluster of four putative effector genes. We confirmed that one of the genes, Avr3D1, encodes an avirulence protein that is specifically recognized by some wheat cultivars. Allele swap experiments demonstrated that polymorphism in the coding sequence is the main cause of the differences in virulence between the two isolates. Population genetics analyses showed that Avr3D1 is present in all of 132 investigated isolates from around the world and that it has evolved under diversifying selection. In contrast, the transposable elements and the putative effector genes surrounding Avr3D1 are under presence/absence polymorphism. Genes in the cluster were shown to be silenced *in vitro* but highly upregulated during infection. Thus, we identified a novel avirulence gene whose recognition leads to quantitative resistance. Its highly controlled gene expression regulation, its clear signs of diversifying selection and its localization in a highly dynamic genomic environment provide us with evidences of the evolution of this avirulence gene to escape recognition.

Ivano Brunner, Institute of Forest, Snow and Landscape Research WSL, Birmensdorf

#### **Jules Favre - a pioneer of alpine mycology**

"Historical datasets of living communities are important because they can be used to document creeping shifts in species compositions. Such a historical data set exists for alpine fungi. From 1941 to 1953, the Swiss scientist Jules Favre visited yearly the region of the Swiss National Park and recorded the occurring fruiting bodies of fungi >1mm (so-called "macrofungi") in the alpine zone. Favre can be regarded as one of the pioneers of alpine fungal ecology not least because he noted location, elevation, geology, and associated plants during his numerous excursions. However, some relevant information is only available in his unpublished field-book. Overall, Favre listed 204 fungal species at 26 sampling sites, with 46 species being previously unknown. The analysis of his data revealed that the macrofungi recorded belong to two major ecological groups, either they are symbiotrophs and live in ectomycorrhizal associations with alpine plant hosts, or they are saprotrophs and decompose plant litter and soil organic matter. The most frequent fungi were members of *Inocybe* and *Cortinarius*, which form ectomycorrhizas with *Dryas octopetala* or the dwarf alpine *Salix* species."

Salome Schneider (Group Rigling), Swiss Federal Research Institute WSL, Birmensdorf

#### **High throughput monitoring of *Dothistroma* and *Lecanosticta* needle blight in Switzerland**

Both *Dothistroma* and *Lecanosticta* needle blight belong to the most damaging foliage diseases in natural pine stands and plantations worldwide. The needle blights are caused by three fungal species, which are registered as quarantine organisms in Europe: *Dothistroma septosporum*, *D. pini* are responsible for the *Dothistroma* and *Lecanosticta acicola* for the *Lecanosticta* needle blight. The infection of the needles results in reduced tree growth and consequently low biomass productivity or even death of the trees. For both needle blight diseases the trees show similar symptoms and the pathogens causing *Dothistroma* needle blight can be clearly segregated using DNA-based techniques only. First reports of the diseases in Switzerland date from 1989 for *Dothistroma* and 1995 for *Lecanosticta*. The aim of this study was to obtain an overview on the abundance and spatial distribution of the three pathogens in forests as well as urban areas in Switzerland. With quantitative PCR, the abundance of all three quarantine pathogens was monitored on needles collected at almost 400 locations with five trees each in 2016. In addition, collected needles were screened morphologically for the presence of fruit bodies or spores. Automated DNA extraction and multiplex quantitative PCR allowed for fast and reliable screening of around 1700 trees, of which 10% were positive for one or more of the needle blight pathogens. Among the three diagnosed species, *D. septosporum* was the most common with 17% of all locations, followed by *L. acicola* (8.5%). In contrast, *D. pini* was found only once in canton Bern. In conclusion, the three quarantine pathogens are present in Switzerland and phytosanitary measures need to be defined to avoid further increase of the diseases and their impacts on pine trees.

Banerjee Samiran Agroscope (Group van der Heijden) Agroscope, Zürich

**Impact of land-use intensity on root fungal network complexity and the abundance of keystone taxa**

Agricultural intensification combined with high tillage, monoculture, and excessive use of fertilizer, pesticides and insecticides often resulted in degraded and less fertile soils. Alternative cropping practices such as reduced tillage, improved crop rotation and organic farming are widely adopted with an aim of alleviating agricultural impact on the environment. However, it is hitherto unclear how such cropping practices may affect root microbial communities in comparison to intensive conventional farming. This is particularly important since microbes play key roles in a wide range of ecosystem services. This project aimed to unravel such impact by assessing soil and root samples from 60 agricultural farms in Switzerland. Samples were collected from three land-uses: conventional, organic and no-tillage (20 each). Comprehensive edaphic characterization and biological properties were conducted. Composition and diversity of fungi in wheat root were assessed using PacBio SMRT Sequel Sequencing. Our analyses showed that root fungal composition and structure were significantly ( $P < 0.05$ ) affected by land-use intensity. The overall root biomass was also significantly higher ( $P < 0.05$ ) in organic farming systems. Similarly, root fungal groups such as Agaricales, Paraglomerales and Glomerales were significantly different across three management practices and mycorrhizal colonization of wheat roots was on average 50% higher in organic farmlands. Root fungal communities exhibited three distinct clusters based on management practices, with bulk density, pH and total phosphorus content as the edaphic determinants. Network analysis revealed that conventional farmlands harbored simple network with fewer interactions and no keystone taxa, whereas organic systems harbored a much diverse network and many keystone taxa. Overall, our results indicate that land-use intensity alters root fungal network structure, and that organic farming enhances network complexity and the abundance of keystone taxa.