Zürich Mycology Symposium 2022 – Physical part

10th June 2022 – University of Neuchâtel, Faculté des Sciences, Rue Emile-Argand 11, 2000 Neuchâtel (find your way). Room F200 (2nd floor).

10h00-10h30	Arrival and registration
10h35-10h45	Welcome address
10h45-11h00	**Ludwig Beeken (WSL, Swiss Forest Protection)** - Outline of neomycetes in Switzerland
11h00-11h15	**Wajeeha Shamsi (WSL, Phytopathology)** - Hunting for virulence reducing viruses from the native population of *Hymenoscyphus fraxineus* to control ash dieback in Europe
11h15-11h30	**Mathias Mayer (WSL, Forest Soils and Biogeochemistry)** - Soil fungal communities in a disturbed forest landscape
11h30-11h45	**Ferran Romero Blanc (Agroscope, Plant-Soil Interactions)** - Microbiome manipulation in experimental microcosms reveals strong relationships between soil biodiversity and agro-ecosystem functioning
11h45-12h00	**Matteo Buffi (Unine, Microbiology)** - Fungal drops: a novel method for the observation of fungal modularity and coordination
12h00-14h00	Posters-lunch
14h00-14h15	**Benjamin Dauphin (WSL, Ecological Genetics)** - Genomic determinants of ectomycorrhiza formation and transcriptomic imprinting of symbiosis genes in a basidiomycete with poplar
14h15-14h30	**Dominique Sanglard (CHUV, Microbiology)** - Azole resistance in *Aspergillus fumigatus* from swiss soils: a nation-wide study
14h30-14h45	**Marcel van der Heijden (Agroscope & UZH)** - Pesticides, Beneficial Fungi and Soil Health
14h45-15h30	**PODIUM DISCUSSION - Daniel Rigling (WSL, Phytopathology) and Dominique Sanglard (CHUV, Microbiologie)** – The past, present and future of mycology in Switzerland and abroad
15h30-16h00	Open discussion and concluding remarks
From 16h00 onwards	Apero and barbecue under the pine trees

Outline of neomycetes in Switzerland

Ludwig Beenken, Jonas Brännhage, Andrin Gross

Swiss Forest Protection, WSL Birmensdorf

With the increase in global trade, the number of introduced fungal species (neomycetes) is also rising exponentially. Around 300 neomycetes are now known in Switzerland. A third of them come from North America and almost 20% from Asia. However, the origin of a quarter of the neomycetes in Switzerland is unknown. It is remarkable that almost 80% of the neomycetes are parasites. Among these, powdery mildew fungi are the largest group with 20% of all neomycetes, followed by rust fungi and downy mildew with 12% each. Most neomycetes occur in cultivated land and urban areas, but 32% originate from forests. Some of these fungi are invasive tree pests and cause tree diseases that pose a threat to the survival of certain tree species and the native organisms associated with them. Examples of this are ash dieback and chestnut blight. Other foreign pathogens have newly arrived in Europe and Switzerland in recent years. For Example: *Basidiophora simplex* on *Symphiotrichum novae-angliae*, *Eutypella parasitica* on *Acer* spp., *Erysiphe corylacearum* on *Corylus avellana* and *Erysiphe salmonii* on *Fraxinus* spp. With some fungi, however, it is also not clear whether they are real neomycetes or native fungi that have only been overlooked so far. Examples are: *Cryphonectria carpinicola* sp. nov. on *Carpinus betulus* and *Petrakia liobae* sp. nov. on *Fagus* spp. Furthermore, we present an as yet undescribed ascomycete that has been recently found on the pollen cones of spruce in the Alps.

Hunting for virulence reducing viruses from the native population of *Hymenoscyphus fraxineus* to control ash dieback in Europe

Wajeeha Shamsi, Simone Prospero

Phytopathology, WSL Birmensdorf

Plant pathogenic fungi have been reported to harbour mycoviruses that reside either in their cytoplasm or mitochondria. Unlike other viruses, mycoviruses generally lack an extracellular transmission route and can spread vertically via spores to the progeny and horizontally via hyphal anastomosis to other fungal isolates. Mycoviruses have been reported in all major groups of fungi and are gaining increasing attention as biocontrol agents against plant diseases, since some of them can reduce virulence in their host (e.g. CHV1 in the chestnut blight fungus *Cryphonectria parasitica*). Since the last two decades *Hymenoscyphus fraxineus*, the causal agent of lethal ash dieback, has spread across Europe. This pathogen was likely introduced from Asia, where it occurs as a saprophyte. In the present study, total RNA of 116 *H. fraxineus* isolates from Japan were sequenced by using the Illumina RNA-seq platform. Five mycoviruses were identified using *in silico* mining of publicly available genomic data and confirmed through RT-PCR. Four viruses belong to the recognized families Partitiviridae, Endornaviridae, Narnaviridae and genus *Botybirnavirus*. Additionally, a negative stranded RNA virus was identified. A mitovirus was transmitted to virus-free isolates by using the paired culture technique and effects of virus on the host fungus were investigated. Furthermore, we assessed virulence of virus-free and virus-infected isolates, infected with mitovirus, on European ash (*Fraxinus excelsior*) trees by conducting inoculation experiment in the greenhouse.

Soil fungal communities in a disturbed forest landscape

Mathias Mayer, Ivano Brunner

Forest Soils and Biogeochemistry, WSL Birmensdorf

Climate change is expected to increase the frequency and severity of forest disturbances, including windthrows, bark beetle attacks, or fires. Altered disturbance regimes can have far-reaching consequences for biodiversity, due to effects on forest structure, composition, and functioning. This talk focuses on the so far overlooked impact of disturbances on forest soils and demonstrates highly sensitive responses of fungal communities, with ectomycorrhizal fungi being particularly affected.

Microbiome manipulation in experimental microcosms reveals strong relationships between soil biodiversity and agro-ecosystem functioning

Ferran Romero Blanc

Plant-Soil Interactions, Agroscope Reckenholz

Modern agriculture is a major cause of soil degradation. The intensive use of agrochemicals (e.g., pesticides and fertilizers) boosts short-term productivity in terms of plant yields but it can reduce soil biodiversity. There is an increased awareness that soil life plays a crucial role as it drives a myriad of functions that ensure the provision of ecosystem services (e.g., food provision, climate change mitigation). Here we present the results of two independent studies where we manipulated the composition and diversity of biological communities in soil microcosms to test whether changes in the soil microbiome influence soil multifunctionality including plant biomass production (Allium porrum). Additionally, we exposed manipulated and non-manipulated soils to fertilizers (experiment one) and pesticides (experiment two), to further explore how different levels of soil biodiversity interact with agrochemicals. Our experimental manipulation achieved a significant reduction of soil alpha-diversity (46% reduction in bacterial richness, 83% reduction in eukaryote richness), including the complete removal of key taxa (i.e., arbuscular mycorrhizal fungi). This reduction in alpha diversity led to an overall decrease in soil multifunctionality, with the strongest alterations observed in aboveground biomass, nutrient leaching, and carbon use efficiency. Agrochemical application had little effect on ecosystem functions compared to microbiome manipulation. Our results suggest that soil community composition is a key factor regulating soil multifunctionality. Therefore, changes in soil communities (i.e., reductions in alpha-diversity) threaten ecosystem multifunctionality and the services that rely on it.

Fungal drops: a novel method for the observation of fungal modularity and coordination

Matteo Buffi, Saskia Bindschedler, Pilar Junier

Laboratory of Microbiology, University of Neuchâtel

Soils are highly heterogeneous ecosystems at the microscale. Water patches, solid particles, and air gaps are inter-mixed forming a dynamic matrix where a large variety of organisms thrive and interact. In soil, filamentous fungi need to coordinate their mycelia to cope with these varied stimuli. Their exploratory nature combined with their modularity, makes them perfectly adapted to successfully colonize this environment. In this study, we developed an inexpensive and fast method to study and quantify modularity in filamentous fungi at the mycelial and hyphal scales. By depositing 15-20 µL droplets on a slightly hydrophobic surface, a patchy and heterogeneous environment separated by air-gaps is recreated, akin to a 2D soil-like structure. A fungus (spores or mycelia) is inoculated in one of the drops, from which mycelium will emerge and explore its surroundings. Fungal response can be

assessed at different scales by stereoscopy and inverted microscopy images. The latter can be used for fractal dimension analyses of the hyphal network. With this approach we were able to describe mycelial architecture in response to various environmental stimuli. This new method could be useful to disentangle the mechanisms behind mycelium modularity and prompt response to different stimuli in filamentous fungi. In addition, it is fast, accessible, and allows for a high level of replication.

Genomic determinants of ectomycorrhiza formation and transcriptomic imprinting of symbiosis genes in a basidiomycete with poplar

Benjamin Dauphin

Ecological Genetics, WSL Birmensdorf

Forest ecosystems are made up of a wide diversity of organisms that interact at all levels of biological organisation, from genes to communities. In resource-limited environments, trees have associated with mycorrhizal fungi to facilitate the uptake and exchange of nutrients and water as well as carbon elements produced by photosynthetic activities. As such, the emergence of these symbiotic interactions was a major evolutionary innovation for plants and fungi. However, although a large number of fungal genomes have recently been sequenced, the molecular mechanisms underlying ectomycorrhizal traits remain poorly understood. Here, we investigate the basidiomycete *Pisolithus microcarpus*, an ectomycorrhizal fungus that has the ability to form such relationships with eucalyptus trees as well as non-host trees under experimental conditions. Using a unique collection of 41 monokaryons, the parental dikaryon and five dikaryons formed by spontaneous crosses between monokaryons, six ectomycorrhizal traits were measured including mycorrhization rate, which revealed a wide phenotypic variation in the proportion of poplar roots colonised by the different monokaryons, ranging from incompatible to fully compatible strains. Although originating from the same fruitbody, ectomycorrhizal traits of poplar clones inoculated with these strains exhibited contrasting responses, suggesting a genetic role underlying the signaling pathway of these symbiotic organs. Hence, we conducted a genome-wide association study and found key gene variants in *P. microcarpus* that are involved in ectomycorrhizal traits. Our results also revealed high genetic recombination among monokaryon progeny and random allele sorting at four of the eleven mating type loci known to date. In parallel, we performed gene expression analysis to compare functional responses between compatible and incompatible strains with poplar and found lifestyle-specific transcriptomic profiles for ectomycorrhizal and free-living mycelium tissues. Our study provides a better understanding of the genetic basis underlying ectomycorrhizal symbioses and thus the functioning of forest ecosystems.

Azole resistance in *Aspergillus fumigatus* from swiss soils: a nation-wide study

Dominique Sanglard

Institut de Microbiologie, CHUV Lausanne

Aspergillus fumigatus causes infections in human that are associated with high mortality. These diseases are treated with azole antifungals as principal agents; however azole resistance is now reported and thus challenges alternative therapies.
A. fumigatus is also widely found in the environment and participates to the metabolization and recycling of organic materials in soils. In the environment, *A. fumigatus* is accidently exposed to azoles used in the agriculture and crop protection. Expectedly, *A. fumigatus* in the environment can acquire azole resistance principally by target mutations in Cyp51A (L98H) coupled with Cyp51A promoter tandem repeats (TR34). The occurrence of *A. fumigatus* azole resistance in the environment in Switzerland is not well known and we therefore undertook a nation-wide prospection for *A. fumigatus* azole resistance in diverse soils including those exposed and non-exposed to agricultural activity.

From a total of 327 sample sites gathered in several geographical sites, 145 isolates were recovered, among them 18 were *A. fumigatus* exhibiting resistance to azoles (12.5.% positivity). Genetic analysis in these isolates revealed the signature of azole resistance in Cyp51A by TR34/L98H (14 isolates), but also by TR46/Y121F/T289A (4 isolates). Azole resistance could not be directly associated with the use of azoles from agricultural practice; however, soil samples in which azole-resistant isolates were identified contained traces of agricultural azoles. In addition, some soil samples from natural sites (no agricultural activity) contained azole-resistant isolates, thus suggesting dispersion of azole resistance from unknown sources.
In conclusion, this study confirmed the presence of azole-resistant *A. fumigatus* in soil samples in the swiss territory. Given that *A. fumigatus* can disperse to human, these results represent a potential threat to the at-risk patient population.

Pesticides, Beneficial Fungi and Soil Health

Marcel van der Heijden

Agroscope Reckenholz & University of Zürich

Pesticides are widely used to combat disease and pests and to secure food production. It is still poorly understood whether pesticides influence soil health and soil biodiversity. In order to study this, we investigated the occurrence of 46 synthetic pesticides in 120 agricultural fields in Switzerland. We found pesticides in any field investigated. Also, after 20 years of organic farming without application of synthetic pesticides, we found up to 16 different synthetic pesticides. We even detected traces of pesticides in selected soil samples collected from Antarctica. In a next step, we tested whether the occurrence of pesticides is linked to soil biota and soil functions. We observed that the abundance of a common group of beneficial soil fungi, the arbuscular mycorrhizal (AM) fungi (AMF), was negatively linked to the number of pesticides in the soil. Moreover, the application of fungicides in agricultural fields across Europe reduced AM fungal richness and the ability of AMF to acquire nutrients for plants. Thus, the application of specific pesticides could hamper soil health, soil biodiversity components and the natural ability of soils to feed plants with nutrients. We further observed that pesticide residues in the soil are a critical driver of the soil microbiome influencing the abundance of a wide range of fungal and bacterial taxa. Further studies need to investigate these unexpected consequences of pesticide use for agricultural production and natural soil processes.

Predicting fungal communities from soil properties

Natacha Bodenhausen

Department of Soil Sciences, Research Institute of Organic Agriculture FiBL

Inoculating soils with microbes presents an alternative to chemical fertilizers to increase agricultural sustainability. However, inoculation success varies from field to field and depends on the local microbial community. The local soil microbial communities typically remain unknown while the physico-chemical data of agricultural fields are often available or easy to obtain. In this study we specifically investigated whether it is possible to predict the composition of soil fungal communities based on physico-chemical soil data. We sampled 59 fields used for cereal production and assembled paired data of physico-chemical soil properties as well as profiles of soil fungal communities. Fungal communities were characterized using SMRT sequencing of the entire ribosomal internal transcribed spacer. We used redundancy analysis (RDA) to combine the physical and chemical soil measurements with the fungal community data and identified a set of 10 soil properties that explained fungal community composition. Soil properties with strongest impact on fungi included pH, potassium and sand. Finally, we evaluated the RDA model using leave-one-out validation. Prediction of community composition was successful for most soils, with Pearson correlation coefficients between observed and predicted communities >0.7 for more than half of the fields. We showed that prediction strength was negatively related to community evenness. Future research is now needed to test whether predictions of local soil microbial communities can increase the reliability of field inoculations with microbes.

A thousand-genome panel retraces the global spread and adaptation of a major crop pathogen

Alice Feurtey

ETHZ

Human activity has a tremendous impact on the evolutionary trajectories of many species worldwide. Global trade of agricultural goods contributes to the dispersal of domesticated species but also of their pathogens, reshaping species distributions to include new environments and climates. In the agricultural context, humans control the evolution of crop species as well as some environmental variables, thus imposing selective pressure on crop pathogens. Understanding how pathogens surmount control strategies and cope with new climates is crucial to predicting the future impact of crop pathogens in a changing world. *Zymoseptoria tritici* is a major bread and durum wheat pathogen, reported in most wheat-growing regions of the world, and causes significant damage in wheat cultivation. Using more than a thousand isolates of *Z. tritici*, we assembled the largest-to-date number of genomes from a fungal plant pathogen species in order to retrace its worldwide invasion routes and ongoing genetic exchange among major wheat-growing regions. By reconstructing the evolutionary history of the species, we elucidate its impact on adaptive genetic variation and on the evolution of the pathogen genome. Finally, we identify standing variation for adaptation to new climates encountered during the global spread. Taken together, our work demonstrates how a comprehensive panel of genomes enables deep insights into the evolutionary history of a major crop pathogen.

Abundance of indigenous *Metarhizium* populations in Swiss grassland soils

Noemi Küng

Agroscope Reckenholz

Entomopathogenic fungi are commercially used as biocontrol agents (BCA) against diverse insect pests. Strains of the genus *Metarhizium* are known to infect and kill soil dwelling larvae of scarab beetles.

Metarhizium strains have recently been tested as potential BCAs against the larvae of the polyphagous beetle *Popillia japonica*, which has infested regions in northern Italy and Ticino, Switzerland. Application of well-established fungal BCAs have revealed that densities of up to 10e4 CFUs per gram soil are required to achieve effective larval control. Potential drivers of fungal establishment include sufficient host-abundance as well as abiotic and biotic soil factors. To decipher critical soil factors with relevance for establishment of *Metarhizium*-based BCAs, in this study we analyzed factors that influence the abundance of indigenous *Metarhizium* in soil. *Metarhizium* abundance was assessed in samples of 72 Swiss grassland sites, which were characterized in a previous EU-project (BIOINVENT). From this project, we received a large dataset on soil physicochemical parameters, weather conditions, vegetation composition, and microbial community meta-barcode sequences. *Metarhizium* abundance was determined, using a qPCR approach, which allows quantification of a group of closely related *Metarhizium* species (clade1). The qPCR yielded 10e4 to 10e8 of ITS copies per gram soil (~ 10e2-10e6 CFUs) for the different sites. In parallel, we classified fungal ITS amplicon sequences to the level of sequence variances (SVs). We found a strong correlation ($R = 0.82$, $p < 2.2e-16$) between *Metarhizium* clade1 abundance (qPCR) and cumulated sequence numbers of SVs assigned to Metarhizium clade1. In a next step, the abundance of *Metarhizium* (SVs, qPCR) in the soil will be correlated to abiotic and biotic factors of the sampling sites. This will provide a profound understanding of factors that drive *Metarhizium* abundance and thus contribute to development of biological control strategies against *P. japonica*.

Synchronizing the rhythms of symbiosis: The circadian rhythm between arbuscular mycorrhizal fungi and plants

Soon-Jae Lee, Ian Sanders

Department of Ecology and Evolution, University of Lausanne

Circadian clocks are endogenous timing mechanisms that orchestrate rhythmic behavior and gene expression in organisms. Nearly all organisms have evolved a circadian clock allowing them to adapt to periodic day-night fluctuations. Plants are surrounded by diverse microbiota with which they coevolved, forming the so-called holobiont. Integrated regulation of the holobiont circadian rhythm is important to coordinate shifts in activity over time for all partners. Therefore, understanding the circadian system is crucial for studying fundamental mechanisms linking different components that function as a holobiont. The arbuscular mycorrhizal (AM) symbiosis, formed by plant roots and AM fungi, is one of the oldest and most widespread associations between organisms. By mediating the nutritional flux between plants and many soil microbes, the AM symbiosis could constitute the backbone of the plant holobiont. However, although both of partners of the AM symbiosis are reported to have circadian clocks, not much is known about the phenotypic rhythmicity and circadian gene expression of the symbiosis. We investigated the circadian rhythm of phenotypic traits and transcriptomes in the symbiosis between *Medicago truncatula* and the model AM fungus, *Rhizophagus irregularis*. We demonstrate that presence of *R. irregularis* influences *M. truncatula* leaf nyctinasty, plant “sleeping behavior”, and circadian gene expression. We present the first evidence that circadian oscillations occur at the site of nutritional exchange in the AM symbiosis and identify the circadian genes of *R. irregularis*. This study allowed us to establish a circadian transcriptome atlas of the AM symbiosis.

Evolvability potential and constraints for a major wheat pathogen under fungicide stress

Cecile Lorrain

Plant Pathology – USYS, ETHZ

Evolvability of an organism – the ability to adapt rapidly – requires genetic variability. However, the generation of new variants is ultimately constrained by deleterious effects resulting in a trade-off between evolvability and stability. In eukaryotes, genetic variation caused by single mutations or structural variants, including transposable elements (TEs), can be deleterious, neutral, or beneficial. We aim to unravel a major pathogen's evolvability potential and constraints in response to stress and investigate the prevalence of adaptative single mutations and TE variants during stress adaptation in an emerging evolutionary and fungal biology model: the wheat pathogen *Zymoseptoria tritici*. *Z. tritici* is known for its high genetic diversity and remarkable evolutionary potential, adapting rapidly to the agricultural use of fungicides. We performed an evolution experiment with a panel of eight strains coming from the Middle East, Europe and America, exposed to increasing fungicide concentrations in liquid cultures for fourteen weeks. To estimate potential convergent fungicide adaptation, we combined phenotyping of fungicide resistance and whole-genome sequencing of both pre- and post-evolved lines. We found mutations in expected fungicides targets such as CYP51 and succinate dehydrogenases genes. We also found mutations in transporter genes, especially in highly resistant strains, suggesting a combination of different resistance mechanisms. Both mutation and transposition rate variation seem to be driven by the genetic background rather than fungicide stress, suggesting evolvability variation among *Z. tritici* strains. Altogether, we demonstrate the impact of both mutations and structural variants on evolvability for a major wheat pathogen during stress adaptation.

Optimization of *Ustilago nuda* detection in barley seedlings

Cecilia Panzetti, Eveline Jenny, Karen E. Sullam

Agroscope Reckenholz

Prior to the widespread use of fungicidal seed treatments, cereal seed-borne diseases caused substantial economic losses, and they remain a great concern in organic farming. Given the desire to reduce synthetic chemical plant production products, better tools are needed to evaluate the effectiveness of resistance breeding or biological seed treatments, particularly for internally located seed-borne diseases, such as *Ustilago nuda* (loose smut) in barley. The growth of *U. nuda* is symptomless in barley seeds and plants until its teliospores develop at ear emergence. Previously published visual and molecular detection methods are laborious and/or yield a non-target product. We investigated methods to improve *U. nuda* in seedlings by optimizing seedling growth and qPCR conditions. In order to test how these protocol alterations may affect *U. nuda* detection, seedlings were grown out from infected seeds that were either untreated or treated with warm water, an effective alternative seed treatment against *U. nuda*. The quantification using newly designed COX3 primers appeared to be more sensitive than previously published ITS primers. We found that a greater quantity of *U. nuda* DNA was detected after longer seedling growth periods. The less laborious seedling growth conditions, on filter paper and without light, yielded similar *U. nuda* DNA quantities. From this work, the methodology to determine the presence of *U. nuda* in seedlings, and therefore, the effectiveness of seed treatments or resistance breeding, can potentially be optimized by streamlining seedling growth procedures and using COX3 as the target gene.

Heterologous expression of *Hanseniaspora* sp. transporters in *Saccharomyces cerevisiae* confirms their activity as pantothenate symporters, used by the yeast to obtain this vitamin from other

Maria Paula Rueda Mejia

Agroscope Wädenswil

The genus *Hanseniaspora* is formed by cosmopolitan yeasts that are found in association with plants. In a screen of 40 naturally occurring yeasts, one *Hanseniaspora* sp. isolate (APC 12.1) was identified as a strong fungal antagonist against a variety of plant pathogens.
While testing the interactions between plant pathogenic fungi and our *Hanseniaspora* isolate in different minimal media, it was observed that it grew only in proximity of other fungi or plant roots. Meanwhile, the same experiment conducted in yeast nitrogen base and potato dextrose media showed normal growth. Revising and testing the components of the different media, we found that calcium panthotenate is essential for the growth of the yeast, while the absence of biotin or folic acid had negative effects on colony size.
A search in the genome of *Hanseniaspora* sp. (APC 12.1) revealed six predicted pathotenate transporters (named PANT1-6) and the lack of critical enzymes in the pantothenate biosynthetic pathway. In *S. cerevisiae*, the panthotenate synthase Pan6 is required to produce this vitamin, while the plasma-membrane symporter Fen2 transports it into the cell. Employing a pan6 mutant, we constructed a strain with FEN2 under the inducible GAL.L promoter. This strain obtains panthotenate from the medium when galactose is present, growing comparably to the wild type, but shows a strong growth defect in media with glucose as carbon source. With the goal of confirming and describing their function, we used this strain for heterologous expression of the six putative pantothenate transporters. Of the six genes, PANT2 and PANT4 expression rescued normal growth in media without galactose, supporting their function as pantothenate transporters. These results show that *Hanseniaspora* (APC 12.1), though metabolically limited, has mechanisms to obtain essential nutrients from neighboring organisms, which likely supports its fast growth and successful antagonism of other fungi.

Elucidating the biosynthesis of ribosomally synthesized, backbone N-methylated macrocyclic peptides

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Institute of Microbiology, ETHZ

Backbone N-methylation and macrocyclization are desired modifications of peptide therapeutics, as they are known to improve cell permeability, target selectivity and proteolytic stability of peptides. The most famous example of a peptide therapeutic with these modifications is cyclosporin A, a non-ribosomal peptide produced by the fungus *Tolypocladium inflatum*, which is used as an immunosuppressant. Other peptide natural products displaying backbone N-methylation are the borosins, a new family of ribosomally synthesized and post-translationally modified peptides (RiPPs). The precursor proteins of these peptides contain a characteristic SAM-dependent peptide α-N-methyltransferase domain, which iteratively methylates the core peptides located at their C-termini. After completion of methylation, the borosin core peptide is cleaved off and, at least in some cases, macrocyclized by specific endoproteinases. The founding members of this RiPP family are the omphalotins, 12-amino acid macrocycles with nine backbone N-methylations. These peptides are produced by the mushroom *Omphalotus olearius* and are known to exhibit strong toxic activity against nematodes. The mushrooms *Lentinula edodes* (Shiitake) and *Dendrothele bispora* produce highly homologous peptides referred to as lentinulins and dendrothelins. The biosynthetic gene clusters of these peptides encode not only the respective peptide precursor proteins but also a conserved prolyl oligopeptidase and several additional enzymes that are not yet characterized but thought to be involved in the biosynthesis of these peptides. We established the heterologous expression of the precursor protein and the prolyl oligopeptidase in

the yeasts *Pichia pastoris* and *Saccharomyces cerevisiae*, which was found to be sufficient for the production of the peptides in these hosts. In vitro experiments with the purified precursor protein and prolyl oligopeptidase, however, suggest that the prolyl oligopeptidase is not able to process the full-length precursor protein and that additional enzymes are involved in this process. We are aiming at the identification of these enzymes to further shed light on the biosynthetic pathway of these peptides and pave the way towards the biotechnological production of novel backbone N-methylated macrocyclic peptides with advantageous pharmacological properties.

The functional ecology of plant microbiome interactions linked to the wheat plant pathogen *Zymoseptoria tritici*

Luzia Stalder, Daniel Croll

Laboratory of Evolutionary Genetics, University of Neuchâtel

Plant-associated microbiomes promote plant health in natural environments and can confer resistance to pathogens. Within these microbial networks, plant-beneficial and plant pathogenic strains are often closely related. Hence, monitoring potential pathogenic and plant beneficial microbes at the strain level is critical for our understanding of microbiome functions. However, comprehensive strain level monitoring has been challenging. Here, we characterize strain level microbiome interactions of *Zymoseptoria tritici*, the main pathogenic fungus of wheat. We use the PacBio Sequel II system to sequence newly developed and highly polymorphic amplicons that allow to characterize pathogen strain diversity in environmental samples. We have developed a joint set of amplicons to monitor the bacterial diversity of the Pseudomonas genus, which includes many potential agonistic and antagonistic species. Alongside, we sequence the full-length 16S and fungal ITS loci to generate deep insights into crop microbiomes. We apply our set of amplicons to an extensive hierarchical set of wheat samples spanning the growing season, different plant genotypes, as well as replicated leaf and root compartments. The deep sequencing allows us to track *Z. tritici* strain communities and their microbial environment at very high granularity. We will use evidence for co-occurrence or exclusion of individual genotypes to validate previously unknown synergistic and antagonistic microbiome interactions under controlled conditions. This will enable us to identify biotic and abiotic factors determining the ecological niche of *Z. tritici* and reveal principles of competitive exclusion and persistence of crop pathogens in general. Overall, our work introduces an innovative model system to comprehensively investigate plant microbiome interactions.

The potential of *Pseudomonas* spp. to control ink disease of chestnut

Valentin Troxler

Phytopathology, WSL Birmensdorf

In Europe, sweet chestnut (*Castanea sativa*) is threatened by the ink disease, caused by the oomycetes *Phytophthora cinnamomi* and *P. cambivora*. In Switzerland, the disease mainly threatens the extensive chestnut forests in Ticino, the so called “selve”. Since the use of chemicals is generally prohibited in Swiss forests, alternative treatment methods are needed. One such method is the biological control using antagonistic bacteria. Fluorescent pseudomonads are a group of bacteria which display antimicrobial traits against many plant pathogens in agricultural systems, but their use in forests has hardly been explored. The aim of this master thesis was to isolate *Pseudomonas* spp. from the rhizosphere of chestnut trees in Ticino and then to test them for their in-vitro antagonistic properties against the pathogens *Phytophthora cinnamomi* and *P. cambivora*. For this purpose, the rhizosphere of 30 chestnut trees at six sites in Ticino showing symptoms of ink disease was used for the isolation of the bacteria. The results show that fluorescent pseudomonads are present in the chestnut forests of

Ticino and that they form associations with the roots of these trees. Out of 25 tested isolates from Ticino, five were identified that displayed in-vitro antagonistic properties against *P. cinnamomi* and *P. cambivora*. Split plate assays showed that volatile compounds play a minor role in the inhibition of pathogen growth. In a screening with specific PCRs, the strains did not possess any genes encoding the widely known metabolites involved in antifungal activity (such as for example hydrogen cyanide and phenazines). Phylogenetic characterization of the inhibitory strains is currently ongoing, and will possibly help to understand possible antimicrobial traits. Overall, fluorescent pseudomonads are promising biocontrol agents against *Phytophthora* species affecting sweet chestnut. Further research is needed to test for their efficacy in nursery and field conditions.