

Swiss Confederation

ZURICH MYCOLOGY SYMPOSIUM 2020

January 24th 2020, Agroscope in Wädenswil

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Morning program:

09:00	-	09:05	Opening
09:05	-	09:25	Mónica Garcia Otálora Exploiting a treasure case: Digitization projects of the Zurich Fungarium and their scientific results
09:25	-	09:45	Florian Staubli Genetic patterns of maternal and paternal Burgundy Truffle (<i>T.aestivum</i>) individuals in natural populations
09:45	-	10:05	Artemis Treindl Adaptive evolution and genome structure in fungal grass endophytes
10:05	-	10:40	Coffee break
10:40	-	11:00	Markus Künzler Bidirectional propagation of signals and nutrients in fungal networks via specialized hyphae
11:00	-	11:20	Anik Dutta Empirical evidences support virulence-transmission trade-off hypothesis and cost of specialization in wheat- <i>Zymoseptoria tritici</i> interaction
11:20	-	12:20	Keynote Lecture sponsored by Microsynth: Kenneth H. Wolfe: Origin of yeast's natural genome engineering system, mating-type switching
12:20	-	13:50	Lunch break



Afternoon program:

13:50	-	14:10	Leen Abraham Genome-wide expression analysis to map regulatory polymorphisms in a major fungal pathogen
14:10	-	14:30	Ursula Oggenfuss Invasion routes of transposable elements across the genome
14:30	-	14:50	Orsolya Kedves & György Sipos Microbial biocontrol of Armillaria root rot
14:50	-	15:10	Aboubakr Moradi Coprinopsis cinerea lectin 2 protects plants against fungal pathogen Botrytis cinerea
15:10	-	15:30	Sébastien Bruisson Endophytes and epiphytes from the grapevine leaf microbiome as potential agents against phytopathogens
15:30	-	16:05	Coffee break
			Charlotte Joller
16:05	-	16:25	
	-		The range of action of MMTS, a sulphur containing volatile organic
	-	16:45	The range of action of MMTS, a sulphur containing volatile organic compound with anti-oomycete activityAndreas Bühlmann The apple fruit mycobiome: influence of orchard management, variety,
16:25		16:45	The range of action of MMTS, a sulphur containing volatile organic compound with anti-oomycete activity Andreas Bühlmann The apple fruit mycobiome: influence of orchard management, variety, storage time and storage atmosphere Lea Stauber

ABSTRACTS

Exploiting A Treasure Case: Digitization Projects Of The Zurich Fungarium And Their Scientific Results

Mónica Garcia Otálora (ETH Zurich, Institute of Integrative Biology)

The world's herbaria safeguard some 350 million specimens of plants, algae, fungi and fungus-like organisms that represent a unique source of information about the earth's vegetation. Herbarium specimens and their associated data are not only essential for taxonomy and systematics, but can also be exploited for other disciplines like ecology, conservation or food security. For a long time, herbarium specimens have been accessible only to a small number of scientists. Nowadays, herbaria and other natural history collections work hard to digitize their holdings and share the images and data of the specimens with specialists and lay people around the world. The Zurich Fungarium is an outstanding collection of fungi and fungus-like organisms and keeps about a million specimens from all over the world. The staff of the Fungarium takes part in the global digitization programme and focuses on rust fungi, a group of economically important plant parasitic basidiomycetes.

The distinguishing characters of rust fungi are microscopic so that they cannot be shown by in toto digitization. We are therefore using alternative approaches of digitization to image the full breadth of information associated with our rust fungal specimens: (a) Imaging of the specimens' labels bearing all collection data, and (b) imaging of the distinctive morphological characters from microscopic preparations. Up to now, the labels of the whole rust collection (> 57'000 specimens) have been digitized and the pictures published on the picture platform E-Pics of the ETH library. Our current work is dedicated to digitizing rust fungi at microscopic level following the scientific importance of the specimens (e.g. type specimens) and their relevance for actual taxonomic studies. The digitization at microscopic level has already resulted in: i) the description of two new rust species, one of them a new disease of wild potatoes, ii) synonymizing the names of several rust species, iii) publication of a monograph of the rust species parasitizing plants of the nightshade family, and iv) a catalogue with 2000 macro-photographs and light-micrographs of some 600 specimens of rust on Solanaceae published in E-Pics. These results show that intelligent digitization projects



are not only an important service for science but can directly produce new scientific findings.

Genetic patterns of maternal and paternal Burgundy Truffle (T.aestivum) individuals in natural populations

Florian Staubli (Swiss Federal Research Institute WSL, Biodiversity and Conservation Biology)

The burgundy or summer truffle *Tuber aestivum* Vittad. (syn. *Tuber uncinatum* Chatin) is a heterothallic ascomycete, forming an ectomycorrhizal symbiosis with various host tree species. In order to obtain more insight into its reproductive cycle, the fine scale genetic structure of maternal and paternal genotypes originating from a natural T. aestivum population near WSL Birmensdorf ZH was determined. This study site harbors a single Fagus sylvatica tree colonized by T. aestivum, which is surrounded by grassland. Over a time period of 3 years fruiting bodies were harvested and genotyped using microsatellites and mating type genes. Surprisingly the sampled fruiting bodies formed a fairy ring structure around their host tree and even a radial outgrowth from one year to the other was observed. Genotyping revealed that the whole tree is mainly colonized by one big mother individual of one mating type, undergoing sexual reproduction with a more heterogeneous pool of fathers. In total 2 maternal and 10 paternal genotypes were found. Maternal genotypes were also present as fathers only differing in mating type, suggesting that closely related individuals can reproduce sexually. The results of the present study are in agreement with previous studies, that maternal mycelium is in symbiosis with a host tree, while paternal structures persist in the soil. However, the exact structure of the fathers and how mating works remains unknown. At the moment newly harvested fruiting bodies and ectomycorrhizal root tips are genotyped to support the previous findings. Further mycelium from soil samples will be quantified using a qPCR approach based on the fungal ITS sequence to see if there is a correlation between soil mycelium abundance and fruiting body production.



Adaptive evolution and genome structure in fungal grass endophytes

Artemis Treindl (ETH Zurich, Institute of Integrative Biology)

The host plant constitutes the distinct ecological niche that symbionts evolve in and is the most important selective agent for microorganisms such as fungal endophytes that depend on their living host. Most previous studies on fungal adaptation genomics have focused on host-pathogen systems in agricultural environments, however, here we use natural associations of *Epichloë* endophytes as a model system to shed light on the genetic basis of adaptation and the evolutionary forces shaping genomic diversity.

Epichloë fungi (Ascomycota, Clavicipitaceae) are systemic endophytes that colonize the intercellular space of cool-season grasses and produce epiphytic fruiting bodies on the flowering tillers during sexual reproduction. By combining comparative and population genomic analyses of two sympatric *Epichloë* species specialized on distinct hosts across Western Europe, we found that the genomes of these fungi are strikingly distinct and compartmentalized into conserved, gene-dense regions and repeat-rich, gene-poor regions. The repeat-rich regions evolve more quickly and may provide a source of genetic novelty for adaptive evolution. Furthermore, genome wide patterns of divergence within species indicate high levels of differentiation between populations and distinct evolutionary histories. These findings elucidate how genetic mechanisms contribute to adaptation in plant-associated fungi and help identify selective sweeps that may be involved in adaptation to the host plant. Studies like the one performed here are essential to gain a broader understanding of how plants and fungi coevolve and interact in natural systems, to be able to predict evolutionary trajectories and assess threats posed by fungal diseases.



Bidirectional propagation of signals and nutrients in fungal networks via specialized hyphae

Markus Künzler (ETH Zürich, Institute of Microbiology)

Intercellular distribution of nutrients and coordination of responses to internal and external cues via endogenous signaling molecules are hallmarks of multicellular organisms. Vegetative mycelia of multicellular fungi are syncytial networks of interconnected hyphae resulting from hyphal tip growth, branching, and fusion. Such mycelia can reach considerable dimensions and, thus, different parts can be exposed to quite different environmental conditions. Our knowledge about the mechanisms by which fungal mycelia can adjust nutrient gradients or coordinate their defense response to fungivores is scarce, in part due to limitations in technologies currently available for examining different parts of a mycelium over longer time periods at the microscopic level. Here, we combined a tailor-made microfluidic platform with time-lapse fluorescence microscopy to visualize the dynamic response of the vegetative mycelium of a basidiomycete to two different stimuli. The microfluidic platform allows simultaneous monitoring at both the colony and single-hypha level. We followed the dynamics of the distribution of a locally administered nutrient analog and the defense response to spatially confined predation by a fungivorous nematode. Although both responses of the mycelium were constrained locally, we observed long-distance propagation for both the nutrient analog and defense response in a subset of hyphae. This propagation along hyphae occurred in both acropetal and basipetal directions and, intriguingly, the direction was found to alternate every 3 hr in an individual hypha. These results suggest that multicellular fungi have, as of yet, undescribed mechanisms to coordinate the distribution of nutrients and their behavioral response upon attack by fungivores.

Empirical evidences support virulence-transmission trade-off hypothesis and cost of specialization in wheat-Zymoseptoria tritici interaction

Anik Dutta (ETH Zurich, Institute of Integrative Biology)

The modeling of pathogen evolution and disease epidemics requires a proper understanding of the factors affecting variation in virulence and transmission. The trade-off hypothesis forming the basis of modeling pathogen evolution has been extensively used in animal and insect ecology. Furthermore, trade-offs between pathogens' host range for virulence and overall transmission and vice-versa are predicted to affect the evolutionary trajectory. However, these issues are mostly unexplored in wheat-Zymoseptoria tritici interaction. We used a dataset generated by automatic image analysis from 12 diverse wheat cultivars and 145 fungal isolates to investigate the variation in virulence and transmission, their overall relationships, and the cost of cultivar specialization for a trait. The Individual effect of cultivar, isolate, and interaction all contributed to the trait variation. We found a significant positive correlation between virulence and transmission that supported the trade-off hypothesis. Additionally, cultivar specialization for virulence led to overall reduced performance of transmission and vice-versa. The non-existence of a saturation point on the correlation curve indicated a simultaneous increase in virulence-transmission that can be further aided by monocropping. Additionally, specialization can be beneficial to maximize both traits, but the associated cost might create an evolutionary constraint. Different molecular mechanisms such as antagonistic pleiotropy or mutation accumulation might be associated with cultivar specialization.



Genome-wide expression analysis to map regulatory polymorphisms in a major fungal pathogen

Leen Abraham (University of Neuchatel, Laboratory of Evolutionary Genetics)

In agricultural ecosystems, outbreaks of diseases are frequent and pose a significant threat to food security. For fungal pathogens infecting hosts, gene regulation ensures well-timed gene expression to avoid detection by the host immune system. However, the process of regulatory adaptation is poorly understood in particular at the population level. Here, we used Zymoseptoria tritici, one of the most important pathogens of wheat, to generate a genome-wide map of regulatory polymorphism governing gene expression. We investigated the polymorphisms influencing transcription levels of 146 strains by performing expression quantitative trait loci (eQTL) mapping. We identified cis-eQTLs for 65.3% of all genes and the majority of all eQTL were within 2kb of the transcription start site. The proportion of genes with a mapped eQTL was higher for genes on core chromosomes than accessory chromosomes. We also found that indel polymorphism was more likely to act as a cis-eQTL and had on average a higher effect size than SNPs. Next, we contrasted the amount of cis-eQTL mapped across categories of pathogenicity-related genes. Genes encoding functions to manipulate the immune system of the host (i.e. effectors) were less likely to have a mapped cis-eQTL compared to genes encoding enzymes to degrade host tissues. This may indicate that regulatory variation in effector genes is governed rather by epigenetic factors than sequence polymorphism. Our study identified extensive evidence that single pathogen populations segregate large-scale regulatory variation. Such regulatory polymorphism is likely to fuel rapid adaptation to resistant hosts and environmental changes.



Invasion routes of transposable elements across the genome

Ursula Oggenfuss (University of Neuchâtel, Laboratory of Evolutionary Genetics)

Transposable elements are selfish genomic elements that spread by insertion into novel genomic locations. Most TEs have neutral or deleterious effects on host fitness, but there is increasing evidence that TE insertions can mediate adaptation. A hallmark of an actively spreading TE in the genome is a large array of nearly identical copies. Such TE burst are associated with general genome size expansions in many eukaryotes. Yet most TE copies established in the genome are epigenetically silenced, preventing further transpositional activity. A major gap in our knowledge is how transpositional activity and genomic defences jointly control TE invasion routes. Using a global population genomic dataset of a fungal pathogen of wheat, we identify that a large set of TEs are currently active. Strong purifying selection maintains new TE insertions at very low frequency, yet specific TEs were able to invade individual populations leading to significant variation in TE content. To retrace the expansion of individual TEs, we analysed 19 completely assembled genomes. We show that TEs undergo distinct birth-and-death processes in their spread across the genome, leading to highly uneven copy numbers. In conjunction, our work demonstrates how population-level analyses can recapitulate incipient genome expansions driven by TEs.



Microbial biocontrol of Armillaria root rot

Orsolya Kedves & György Sipos (University of Szeged, Department of Microbiology & University of Sopron, Research Center for Forestry and Wood Industry)

Armillaria species are among the most important root pathogens worldwide, attacking more than 500 host plant species of both gymno- and angiosperms.

Biocontrol agents (BCAs) are environment-friendly alternatives of chemicals to prevent the spread of *Armillaria* in forest soils. Bacteria from genera *Pseudomonas* and *Paenibacillus* produce biologically active metabolites inhibiting fungal growth, while *Trichoderma* species apply competition, antibiosis and/or mycoparasitism against a wide range of fungal plant pathogens.

Armillaria and BCA candidates were collected from soil samples of a damaged Hungarian oak- and a healthy Austrian spruce forest. A. cepistipes and A. ostoyae were found in the Austrian spruce forest, while A. mellea and A. gallica dominated the Hungarian oak stand. 64 Trichoderma strains from 14 species, 29 Pseudomonas strains from 5 species, 5 Paenibacillus strains from 3 species were also isolated. In vitro antagonism and antibiosis experiments revealed the potential of certain Pseudomonas, Paenibacillus and Trichoderma isolates to control Armillaria species. Some of the biocontrol produce selected candidates extracellular proved to polysaccharide-degrading enzymes, siderophores and indole-3-acetic acid, as well as to mobilize phosphorous - plant growth-promoting properties which could also be potentially exploited during the biological control of Armillaria root rot.

A field experiment was carried out by applying two selected *Trichoderma* strains on two-year-old European turkey oak seedlings planted to a forest area heavily overtaken by the rhizomorphs of numerous *Armillaria* colonies. Oak seedlings treated with *T. virens* and *T. atrobrunneum* showed better survival under harsh soil conditions than the untreated controls.

Based on our results, *Trichoderma*, *Paenibacillus* and *Pseudomonas* strains have beneficial properties which could be exploited during the biological control of *Armillaria* root rot.

This work was supported by the Hungarian Government and the European Union (Széchenyi 2020 Programme; GINOP-2.3.2-15-2016-00052).



Coprinopsis cinerea lectin 2 protects plants against fungal pathogen Botrytis cinerea

Aboubakr Moradi (University of Fribourg, Department of Biology)

Plant pathogenic fungi have a significant impact on agricultural production. The plant immune system can recognize the presence of pathogens to trigger defensive responses, such as the production of defensive proteins. However, plant fungal pathogens can deliver effectors (virulence factors) into host plants to suppress individual plant immunity responses. To overcome this potential limitation, we use different classes of defensive proteins such as lectins from the basidiomycete fungus Coprinopsis cinerea. Plants produce several lectins proteins that have an essential role in plant immunity and responses to various biotic stress. Likewise, when C. cinerea is exposed to pathogens and predators, it produces several defensive proteins, including lectins, to inhibit the attackers. Functional analysis revealed the toxicity of C. cinerea's lectins (CCLs) towards the model nematode Caenorhabditis elegans. However, there is no evidence about the potential antifungal activity of the characterized CCLs. Three lectins were selected for expression in Arabidopsis thaliana to explore their antifungal potential. C. cinerea lectin 2 (CCL2)-overexpressing plants exhibit significant resistance toward plant fungal pathogen Botrytis cinerea compared to the wild type plants. Interestingly, the expressed mutant version of CCL2 protein (a point mutation in the carbohydrate-binding site) in plants showed no resistance against B. cinerea. These demonstrate that the antifungal activity of CCL2 is mediated by results carbohydrate-binding ability. However, the mode of action and potential target/s of CCL2 either in the A. thaliana or B. cinerea remains to be demonstrated.



Endophytes and epiphytes from the grapevine leaf microbiome as potential agents against phytopathogens

Sébastien Bruisson (University of Fribourg, Department of Biology)

Plants harbor diverse microbial communities that colonize both below-ground and above-ground organs. Some bacterial members of these microbial communities have been shown to contribute to plant defenses against pathogens. In this work, we characterized the pathogen-inhibiting potential of 78 bacterial isolates retrieved from endophytic and epiphytic communities living in the leaves of three grapevine cultivars. We selected two economically relevant pathogens, the fungus Botrytis cinerea and the oomycete Phytophthora infestans. Our results showed that mycelial inhibition of both pathogens through bacterial diffusible metabolites was more widespread among endophytes than among epiphytes. Six closely related Bacillus strains induced strong inhibition (>60%) of B. cinerea mycelial growth. Among these, five led to significant perturbation in spore germination, ranging from full inhibition to reduction in germination rate and germ tube length. Different types of spore developmental anomalies were observed for different strains, suggesting multiple active compounds with different modes of action on this pathogen. Compared with B. cinerea, the oomvcete P. infestans was inhibited in its mycelial growth by a higher number and more diverse group of isolates. Beyond mycelial growth, both zoospore and sporangia germination were strongly perturbed upon exposure to cells or cell-free filtrates of selected isolates. Moreover, three epiphytes strains inhibited the pathogen's growth via the emission of volatile compounds. The comparison of the volatile profiles of two of these active strains with those of two phylogenetically closely related, inactive strains led to the identification of molecules possibly involved in this volatile-mediated pathogen growth inhibition, including trimethylpyrazine, dihydrochalcone, and L-dihydroxanthurenic acid. This work demonstrates that grapevine leaves are a rich source of bacterial antagonists with strong inhibition potential against two pathogens of high economical relevance. It further suggests that combining diffusible metabolite-secreting endophytes with volatile emitting epiphytes might be a promising multi-layer strategy for biological control of above-ground pathogens.

The range of action of MMTS, a sulphur containing volatile organic compound with anti-oomycete activity

Charlotte Joller (University of Fribourg, Department of Biology)

Recently, S-methyl methanethiosulfonate (MMTS), a sulphur containing volatile organic compound (sVOC) emitted by *Pseudomonas* species naturally associated with potato plants, has been identified as an anti-oomycete agent promising for protection of potato crops from the late blight causing agent *Phytophthora infestans*. Indeed, MMTS showed low apparent phytotoxicity coupled with efficient inhibition of *P. infestans* infection. However, older reports on the toxicity of MMTS suggested it to be a "reactive Sulphur species" with a broad range of action and potentially toxic effects against many non-target organisms.

The aim of the present project was hence to determine the range of action of MMTS. To that end, the direct toxicity of MMTS against different plant pathogenic and beneficial fungi, yeast, bacteria, plants as well as nematodes was investigated. So far evidence shows that MMTS is active against all organisms tested in a dose dependent manner. However, the effect against *P. infestans* appears to be particularly strong. Moreover, the mode of action of MMTS is proposed to be very similar to that of allicin, one of the bioactive molecules of garlic, which has been investigated in depth for its potential in protecting both crop and human health. Therefore, despite its wide range of action, MMTS should still be considered as a particularly promising molecule from the mixture of naturally occurring metabolites emitted by the plant microbiome, in terms of disease control in potato as well as in other crops.



The apple fruit mycobiome: influence of orchard management, variety, storage time and storage atmosphere

Andreas Bühlmann (Agroscope, Product Quality and Innovation)

Microbial spoilage in commercial apple storage facilities can lead to food loss of up to 30% during the storage period. While some of the causal pathogens such as Penicillium spp. and Botrytis spp. are well characterized, others such as Neofabraea spp. and Marssonina spp. are less known due to difficulties in culturing under laboratory conditions. Metagenomics allows the screening of apples for the abundance and dynamics of pathogens and the microbiome as a whole in a culture independent way. Here we aimed at characterizing the total microbiome on the apple fruit after harvest and to elucidate the influence of the growing season, apple variety, orchard management practices and storage atmosphere conditions on the microbiome by means of in vitro cultivation and metagenomics. The results allow for the characterization of infection levels of different pathogens at harvest and, to some extent, the prediction of post storage symptom development. Additionally, information about the community composition allows for the identification of the main factors driving the composition of the microbiome, the change in diversity during the storage period and the identification of beneficial microorganisms that may eventually be applied as biocontrol agents in the future. The diversity of the microbiome was shown to differ significantly between orchard management protocols, variety and growing season. The results show a potential to be applied in the development of novel and the improvement of existing infection models, educate breeders on how the host genotype interacts with the microbial community and inform researchers on how microbial communities change over time. Therefore metagenomic characterization of the microbiome may provide a valuable tool to inform practitioners and researchers on disease risks and prevent post-harvest losses in the near future.



Emergence and diversification of a highly invasive tree pathogen lineage

Lea Stauber (Swiss Federal Reseach Institute WSL, Forest health and biotic interactions)

Invasions are often dominated by one or a small number of genotypes, thus leading to genetic bottlenecks in colonizing areas. Despite low genetic diversity some species have become very successful invaders, but underlying factors are poorly understood. An example for a successful invasion is the recent outbreak of the chestnut blight fungus Cryphonectria parasitica in Southeastern Europe. We investigated the genetic structure of 188 C. parasitica strains, using 17'873 genome-wide single-nucleotide polymorphisms (SNPs). Genotypes showed high levels of diversity with evidence for frequent and ongoing recombination. European populations also exhibit a longitudinal gradient in genetic diversity. We found that the dominant invasive S12 lineage is genetically homogeneous. Our data strongly suggests that S12 emerged from the highly diverse pool of European genotypes rather than a secondary introduction from Asia. The S12 lineage is also predominantly composed of a single mating type and the observed genetic diversity is consistent with mutation accumulation. Despite little or no evidence for recombination, we show experimentally that the lineage retained the ability to reproduce sexually. Overall, our findings show that an invasive pathogen lineage can arise through an intermediary, highly diverse bridgehead population. The combination of genetic and epidemiological evidence suggests that the invasive lineage switched the dominant reproductive mode and gained crucial adaptive mutations favouring rapid expansion.



Abundance and diversity of the entomopathogenic fungus *Metarhizium* spp. (Ascomycota: Hypocreales) in soils of three different Swiss land-use types

Maria del Carmen Fernandez-Bravo (Agroscope, Molecular Ecology)

The fungal genus *Metarhizium* spp. is a worldwide occurring arthropod pathogen, and one of the most important commercial biocontrol agents. Although many aspects of the life cycle of this fungus have been investigated in the past, knowledge on how agricultural factors like crop-type, -management, and -protection may affect its presence and population structure has remained incomplete.

The aim of this project was to investigate abundance and diversity of *Metarhizium* in three land-use types (arable-land, grassland and forest), and to assess how Metarhizium population structure is affected by land-use type and/or various physical, chemical and microbiological parameters.

The study was performed at 30 sites that are part of the Swiss national soil-monitoring-network (NABO), including ten sites each of grassland, arable-land, and forest. In 2016, 349 *Metarhizium* isolates were obtained from three bulk soil samples per site using a selective medium. Their multi-locus-genotypes (MLG) were determined by applying 14 microsatellite markers.

Metarhizium spp. were present in 10, 8 and 4 of grassland, arable-land and forest sites, respectively. Abundance and richness was highest in grassland soils, followed by arable-land and forest. Twenty-four MLGs were detected among the 349 isolates and 14 MLGs were identified as *M. brunneum*, 7 as *M. robertsii* and 3 as *M. guizhouense*, representing 259, 80 and 10 isolates, respectively. Statistical analyses revealed that environmental factors, such as "C:N ratio" and "bulk density" in soil, significantly affected *Metarhizium* communities in the three land-use types.

Results showed that grassland harbors the most abundant and diverse *Metarhizium* populations and soil physical and chemical parameters partially explained the difference among the land-use types. As a next step, we will investigate how additional factors like arthropod diversity and/or plant community composition may affect *Metarhizium* populations in soil.



PARTICIPANTS

Please note: The surnames are hyperlinks to the email address. The first names that are shown in blue and underlined are those participants who are presenting (the first name links to the abstract).

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DIRECTIONS

The Mycology Symposium will take place at Agroscope in Wädenswil (in the lecture hall, shown in green on the map), which can be easily reached by train from Zurich (S2, S8 and IR trains, roughly every 10-15 min). From the train station it is either a short walk (uphill, about 10-15 min) or a bus ride (Bus number 126, until stop "Wädenswil Forschungsanstalt, 5 min; always at x:15 and x:45). For an online version of the map shown below, you may click anywhere on the image.

