Virtual Zürich Mycology Symposium 2021

15th January 2021 - https://ethz.zoom.us/j/91692135743 ; Meeting ID: 916 9213 5743

8h45-9h	Welcome address
9h-9h20	**Meixia Yang (Christoph Scheidegger, WSL)** - Phylogeny of *Lobaria* from the Himalayas and the Hengduan Mountains
9h25-9h45	**Joris A. Alkemade (Pierre Hohmann, FiBL)** - Genetic diversity and pathogenicity of the lupin pathogen *Colletotrichum lupini*
9h50-10h10	**Nikhil Kumar Singh (Daniel Croll, UniNE)** - Tracking crop pathogen lifestyles and virulence evolution using population-level deep-sequencing
10h10-10h40	Virtual Coffee break*
10h40-11h	**Bettina Schmid (Philipp Bosshard, USZ)** - Mycobiome sequencing reveals a high fungal diversity in patients with severe atopic dermatitis
11h05-11h25	**Manon Longepierre (Martin Hartmann, ETHZ)** - Limited resilience of the soil fungal diversity within four growing seasons after soil compaction under different management regimes
11h30-11h50	**Stefan Emler (SmartGene Services, EPFL)** - Centroid-based sequence annotation for more accurate and easier identification of Fungi
11h50-13h30	Lunch break [please connect again latest at 13h25]
13h30-13h50	**Valentin Brühwiler (Valentin Queloz, WSL)** - Who's to blame for *Carpinus* decline – a climate change opportunist or a neomycete?
13h55-14h15	**Ola Abdelrahman (Laure Weisskopf, UniFR)** - Evaluating the potential of Actinomycetes and their metabolites as fungicide alternatives
14h20-14h40	**Aislinn Estoppey (Pilar Junier, UniNE)** - Improved methods to assess bacterial biocontrol on germination of fungal spores or sclerotia
14h45-15h15	Virtual coffee break*
15h15-15h35	**Eva Vogt (Markus Künzler, ETHZ)** - Ribosomal peptides derived from KEX2-processed repeat proteins (KEPs) in fungal physiology, defense and development
15h40-16h	**Francisco M. Gámez-Arjona (Clara Sánchez Rodriguez, ETHZ)** - The role of fungal cellulases during plant infection
16h-16h20	Concluding remarks

** Virtual coffee breaks will be held in different breakout sessions. The idea is to join and leave a discussion topic as you feel; more details on January 15th!*

Phylogeny of *Lobaria* from the Himalayas and the Hengduan Mountains

Meixia Yang[1,2], Christoph Scheidegger[1]

[1] Swiss Federal Institute for Forest, Snow and Landscape Research WSL
[2] Faculty of Sciences, University of Bern

In this study, the diversity of green-algal *Lobaria* (Schreb.) Hoffm in the Himalayas and the Hengduan Mountains regions were evaluated by applying both morphological and phylogenetic approaches. Multi-locus phylogenetic analyses of 768 green-algal *Lobaria* specimens collected between 2016 and 2019 were performed using a three-locus and time-calibrated species-tree approach. Taxonomically, 11 green-algal *Lobaria* species were identified as new to science, while 9 were previously described *Lobaria* species and 1 to be determined. The species differentiated during the Pliocene and Pleistocene. The coincidence of paleoclimatic events with estimated dates of divergence supports a bioclimatic hypothesis for the species evolution in the green-algal *Lobaria*. Molecular phylogenies, a summary of diversity, detailed new species descriptions, and geographical analyses are provided. Special recognition of species with a long evolutionary history, which merit high conservation priority, will be especially critical for preserving geographically restricted endemics from the Himalayas and the Hengduan Mountains, where habitat loss is driving rapid declines.

Genetic diversity and pathogenicity of the lupin pathogen *Colletotrichum lupini*

Joris A. Alkemade[1], M. M. Messmer[1], R. T. Voegele[2], M. R. Finckh[3], Pierre Hohmann[1]

[1] Department of Crop Sciences, Research Institute of Organic Agriculture (FiBL), Frick, Switzerland
[2] Institute of Phytomedicine, University of Hohenheim, Stuttgart, Germany
[3] Department of Ecological Plant Protection, University of Kassel, Witzenhausen, Germany

Members of the *Colletotrichum acutatum* species complex cause disease in many important crops. Most species within this complex have a broad host range. *Colletotrichum lupini*, belonging to clade 1, however, appears to be highly host specific on lupins. Lupin anthracnose caused by *C. lupini* is the most important disease in lupin cultivation worldwide, affecting all economically important lupin species. The disease is mainly dispersed via seeds or by rain splash and even low amounts of initial inoculum can cause total yield loss. We collected 39 *C. lupini* isolates from across the world to get insight in its intraspecific diversity and pathogenicity. Based on multi-locus phylogeny together with morphological characterization, six genetic subgroups and ten morphology types could be distinguished, indicating a higher diversity than previously anticipated. Highest diversity was found in South America, which is also considered to be the center of origin for species belonging to clade 1 of the *C. acutatum* species complex. The majority of isolates (n = 26) belonged to genetic subgroup II, exhibited morphology type A and were found across the globe. Virulence assays were performed on two white lupin (*Lupinus albus* L.) and two Andean lupin (*Lupinus mutabilis*) accessions. This showed a high virulence and broad host spectrum for isolates belonging to genetic subgroup II and are proposed to be the cause of the current pandemic. Strong strain x lupin species/accession interaction effects were found, suggesting the existence of different physiological races within *C. lupini*. This study improves our understanding of *C. lupini* diversity and pathogenicity, providing valuable information for breeding programs and future disease management. More extensive sampling, especially from South America, combined with genome wide sequencing are now fundamental to increase genetic resolution and better understand *C. lupini* phylogeny.

Tracking crop pathogen lifestyles and virulence evolution using population-level deep-sequencing

Nikhil Kumar Singh[1], Thomas Badet[1], Leen Abraham[1,] Emilie Chanclud[1] , Petteri Karisto[2,] Daniel Croll[1]

[1] Laboratory of Evolutionary Genetics, Institute of Biology, University of Neuchâtel, 2000 Neuchâtel
[2] Department of Evolutionary Biology and Environmental Studies, University of Zürich, Zürich

In the arms race between hosts and pathogens, plants evolved the ability to recognize virulence factors and pathogens have repeatedly escaped such recognition. This resulted in rapid evolutionary change in pathogen virulence and host resistance genes. To retrace pathogen lifestyles and the genetic basis of host-pathogen interactions, we deep-sequenced 177 single field isolates of *Zymoseptoria tritici*, a major fungal pathogen of wheat. We then used genome-wide analyses to retrace the impact of sexual and asexual reproduction on the population genetic structure of a single field. We identified an astonishing level of genetic diversity exceeding global diversity levels for many other crop pathogens. Then, we used genome-wide association studies to map genetic variants underlying virulence on a specific wheat cultivar planted in the field. We identified a highly dynamic region consisting of multiple families of transposable elements. We show that the virulence locus has undergoing substantial recent sequence evolution including large segmental duplications and transposable element insertions. In conjunction, our work highlights the power of population-scale analyses of crop pathogens to retrace lifestyles and analyze the genetic basis of virulence.

Mycobiome sequencing reveals a high fungal diversity in patients with severe atopic dermatitis

Bettina Schmid[1,2], Eugen Bersuch[1], Axel Künstner[3], Anke Fähnrich[3], Hauke Busch[3], Martin Glatz[1,2], Philipp P. Bosshard[1,2]

[1] Department of Dermatology, University Hospital Zurich, Gloriastrasse 31, CH-8091 Zurich, Switzerland
[2] University of Zurich, Gloriastrasse 31, CH-8091 Zurich, Switzerland
[3] Institute of Experimental Dermatology, University of Luebeck, Ratzeburger Allee 160, 23538 Luebeck, Germany

Atopic dermatitis (AD) is a multifactorial, chronic relapsing inflammatory skin disease. Characteristics are an impaired skin barrier and an altered skin immune system, which often come along with predominant colonization by *Staphylococcus aureus*. The role of fungi, i.e. the mycobiome, remains poorly investigated although AD patients are frequently sensitized to *Malassezia*, the most abundant fungus on skin. We aim to improve the understanding of the skin mycobiome in AD. Skin swabs of 17 AD patients and 16 healthy controls (HC) were taken from 4 skin sites (antecubital crease, glabella, vertex, and dorsal neck). To assess temporal shifts in the mycobiome, AD patients were sampled at 3 time points (0, 2 and 4 weeks). HC were sampled at 2 time points (0, 4 weeks). We assessed relative abundance of fungal genera and species by amplicon-based next-generation sequencing (NGS) of the fungal ITS1 region. The most abundant fungi at all skin sites were *Malassezia* spp. The species distribution was site-dependent with high abundances of *M. globosa* at the neck and *M. restricta* at the glabella and vertex, and overall lower abundance of *Malassezia* at the antecubital crease. As shown exemplary for the neck (figure 1), patients with severe AD tended to be more frequently colonized with non-*Malassezia* fungi such as *Candida*. In most HCs and patients with mild to moderate AD, the mycobiome was comparable between individuals and stable over time. In contrast, in severe AD the mycobiome was different between individuals and changed over time. In conclusion, patients with severe AD had a high intra- and interpersonal species diversity. We speculate that the impaired skin barrier in severe AD allows colonization with more different fungi than healthy skin. Vice versa, the altered mycobiome may cause activation of the skin immune system leading to inflammation and eczema.

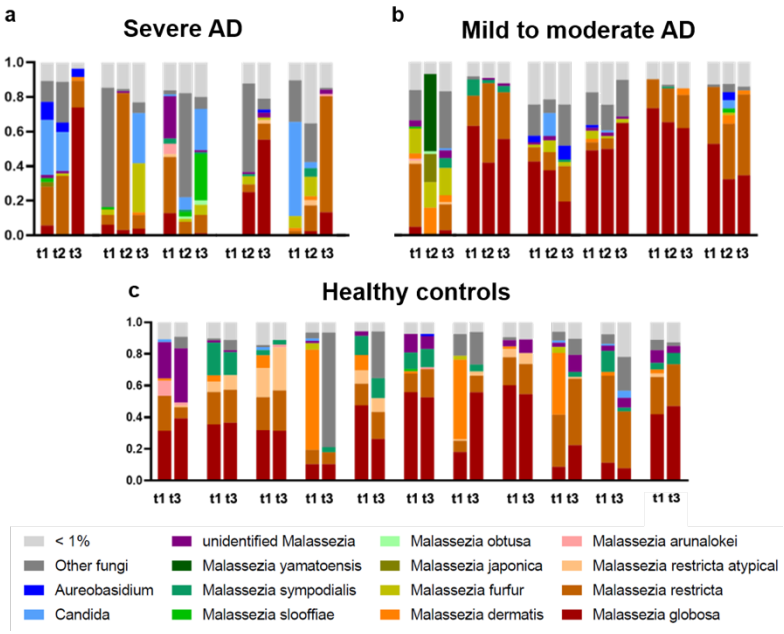

Figure 1: Figure 1: Relative abundances of fungal taxa in swabs collected from the neck in a) severe AD, b) mild to moderate AD, and c) HCs. t1: day 0; t2: day 14; t3: day 28.

Limited resilience of the soil fungal diversity within four growing seasons after soil compaction under different management regimes

Manon Longepierre[1], Franco Widmer[2], Thomas Keller[2,3], Peter Weisskopf[2], Johan Six[1], Martin Hartmann[1]

[1] ETH Zurich, Zürich, Switzerland
[2] Agroscope, Zürich, Switzerland
[3] Swedish University of Agricultural Sciences SLU, Uppsala, Sweden

Soil compaction is a disturbance caused by highly mechanized agriculture, which can constrain soil biodiversity and functioning. Despite the central role of soil fungi in contributing to key ecosystem services such as plant growth, nutrient cycling, and pest control, we currently lack a fundamental understanding of their response to compaction. For this purpose, a long-term soil structure observatory was established in 2014 to monitor recovery of soil structure and function following compaction caused by an agricultural vehicle (32 Mg). The evolution of soil structure and function were assessed under four different post-compaction management regimes that allow studying natural recovery processes with and without vegetation (i.e., perennial grass versus bare soil) as well as restoration under a crop rotation with and without tillage. The response of soil fungal diversity to compaction as well as its resilience after compaction under the different management options was assessed by metabarcoding over a time period of four growing seasons and related to the edaphic background and crop productivity. Soil compaction reduced soil porosity (-15%), air permeability (-96%) and gas diffusivity (-75%). Statistically significant shifts in the fungal community structure were observed up to four growing seasons post compaction (compaction explaining 4% of the variance), showing no complete resilience; however, these effects were much smaller than the spatiotemporal effects observed in the system (explaining 70% of the variance). Fungal taxa that were sensitive or tolerant towards compaction were broadly distributed across the taxonomic tree and present in all major phyla. Saprotrophic fungi such as lignin decomposers tended to increase in compacted soils, whereas plant-associated fungi appeared to be more negatively affected by compaction. Crop yield was reduced after compaction in the first two years (up to 90% reduction in compacted no-till compared to the control conditions), but did largely recover in the following years. Although we found that compaction changed the physical soil properties, caused shifts in fungal community structure and reduced yield in all management systems, the tillage regime appeared to mitigate the compaction effects on all measured topsoil parameters over the studied time period. This study demonstrates that soil compaction, for the majority of the tested post agricultural management, is a disturbance that can have long-lasting effects on soil physics and soil fungal community. Those effects do not necessarily completely align with differences in crop yield. Complementing existing knowledge of agricultural practices with this information is valuable in providing practitioners with recommendations for more sustainable farming systems.

Centroid-based sequence annotation for more accurate and easier identification of Fungi

Stefan Emler[1]

[1] SmartGene Services, EPFL Innovation Park

Identification of fungi by sequencing has become a reference method in laboratories to determine the species present in clinical and environmental samples. To achieve identification, isolate or population sequences of marker genes such as ITS, 18S and D1D2 are usually compared by BLAST to entries in a reference database. Identification of the organism(s) present is based on percentage homology between the sample sequences and reference sequences.

The success of this approach depends greatly on the quality of the reference databases, the accuracy of their annotations and on the extent to which all relevant species and their naturally occurring variants are adequately represented.

The large and ever-growing number of fungal species described creates a significant burden for manual, expert curation, leading to delays in updating and deficient representation within sequence reference databases. To address these issues, SmartGene has developed a Centroid algorithm-based approach for validating the species and genus annotations which are assigned to genetic sequences. This process uses AI and machine learning to extract and filter entries from public data repositories, and to assess the accuracy of the sequences and their annotations:

- In a first step, profile-based extraction methods are applied to extract good quality ITS, 18S, D1-D2. entries from public domain repositories.
- In a second step, entries annotated with a valid species name or synonym are placed in species groups.
- The most representative entry of each species group is determined according to the SmartGene's patented Centroid process;
- a confidence score is computed for the annotation carried by each entry and outlier entries are flagged.
- For each species group, the observed intra-species diversity is used to define species-specific thresholds which enable precise identification of fungi within a sample.
- All Centroid sequences are grouped in a distinct reference database and the scored variants are stored in another.

Sequences or NGS reads from a sample are then rapidly matched against the smaller but complete Centroid dataset; BLAST lists indicate the species group size, synonyms and annotation confidence scores. Differentiation of close species is facilitated and relevant species variants can be retrieved easily. Distance matrices for each genus indicate the potential of differentiation of close species for a given target gene.

The process and databases are regularly updated for newly published data and changing nomenclature and. can be extended to other genes. With each update, the self-learning process improves the knowledge about species diversity and refines the cut-offs used for accurate differentiation. Further validations and improvements are foreseen, in collaboration with a number of Swiss academic institutions.

Centroid-annotated databases at SmartGene, for the identification of fungal isolates and mycobiomes, version Oct 2020

Gene target	# Genera covered	# Species covered	Total number of sequences
ITS	5'018	36'122	471'057
18S	3'522	11'055	56'702
D1-D2	5'415	30'043	149'120
Total	6'437	46'833	

Who's to blame for *Carpinus* decline – a climate change opportunist or a neomycete?

Valentin Brühwiler[1], Ludwig Beenken[1], Valentin Queloz[1]

[1] Waldschutz Schweiz, Eidg. Forschungsanstalt für Wald, Schnee und Landschaft (WSL)

Since the early 2000s, reports of declining hornbeam trees (*Carpinus betulus*), especially in urban areas, are increasing in Italy. The ascomycete *Anthostoma decipiens*, a member of the family Diatrypaceae, has been identified as the causal agent of this dieback. In Switzerland, the first case was reported from the city of Geneva in 2017, with more cases following in Basel shortly afterwards. *A. decipiens* has been known as an indigenous species since the 19th century. The little attention it has received in the literature suggests a saprobic lifestyle. This raises the question as to what might cause this current, apparent increase in aggressivity. One hypothesis is that the fungus profits from host stress caused by summer drought and heat, which are becoming increasingly frequent and severe with climate change, especially in cities. Another possibility may be that the causal agent of the dieback is not actually *A. decipiens*, but a cryptic, more aggressive, introduced relative. In my master's thesis, I collected explorative data to test the plausibility of these hypotheses: observations in the field indicate that *A. decipiens* is not an abundant species in the forest. It appears that it requires slowly perishing trees as hosts and is unable to colonize still healthy or already dead trees. The fungus' reaction to host stress was also assessed experimentally in an infection experiment. Potted young hornbeams were inoculated with six strains of *A. decipiens*. Half of the plants were subjected to a drought stress treatment, while the other half were kept well-watered. After three months, the necrotic lesions, which had developed, were measured. Five of the six strains produced similary sized lesions, with drought leading to a significant increase in lesion area in four of these strains, which indicates that *A. decipiens* may be able to exploit host stress in vivo. The remaining strain, however, showed fundamentally different behaviour: it was overall much less aggressive than the other strains and did not profit from drought stress. The possibility of cryptic speciation thus also remains to be further examined.

Evaluating the potential of Actinomycetes and their metabolites as fungicide alternatives

Ola Abdelrahman[1], Adil El Hussein[2], Marmar El Siddig[2], Sakina Yagi[2], Floriane L'Haridon[1], Laure Weisskopf[1]

[1] Department of Biology, University of Fribourg, Fribourg, Switzerland
[2] Department of Botany, University of Khartoum, Khartoum, Sudan

Yearly, massive yield losses of different crops are happening because of various disease-causing microbes. In order to protect those crops, most producers use repeated applications of synthetic fungicides, which are very harmful to the environment and human health. This project aims at contributing to developing new, environmentally friendly solutions to safeguard plant health, by using Actinomycetes. These Gram-positive bacteria are known for their prolific production of secondary metabolites, among which many natural antibiotics. During my master project, we isolated more than 170 Actinomycetes from the soil and from the rhizosphere of different native plants in Sudan. The aim was to explore the potential of newly isolated Actinomycetes to inhibit different phytopathogenic fungi through the production of volatile and non-volatile metabolites. At first, dual culture Petri dish assays were performed against *Aspergillus niger* and *Fusarium oxysporum*; 54% of the isolates were found to inhibit the mycelial growth of *A. niger* (46%) and of *F. oxysporum* (25%) with variable activity (5 to 19 mm inhibition zone radii). When using split plate Petri dishes allowing only volatiles to reach the pathogens, 50% of the isolates were able to inhibit the growth of *A. niger* (40%) and of *F. oxysporum* (17%). We then selected the most potent 28 isolates and tested their volatile-mediated inhibition against the same two pathogens in addition to *Botrytis cinerea*, *Rhizoctonia solani*, and *Verticillium dahliae*. All of them were very active against *A. niger* with inhibition percentage ranging from 23% to 76%, and against *R. solani* (4 - 78.5%). Twenty-seven isolates were active against *F. oxysporum* (2 – 46.4%), 20 slightly inhibited the growth of *B. cinerea* (0.1 – 20.4%) and four showed very strong activity against *V. dahliae* (44.1 – 70.2%). It is worth mentioning that most isolates had different inhibition specificities of the tested pathogenic fungi but that four of them could inhibit them all. My Ph.D. project investigates the potential of these already isolated Actinomycetes strains to control late blight disease in potato, one of the most devastating pathogens worldwide. Although *Phytophthora infestans* was not included in this initial screening, we are very confident that the metabolites produced by these Actinomycetes will inhibit the oomycete even more strongly than the fungal pathogens, since the sensitivity of oomycetes to antimicrobial compounds, and especially to volatiles is known to be particularly high.

Improved methods to assess bacterial biocontrol on germination of fungal spores or sclerotia

Aislinn Estoppey[1], Laure Weisskopf[2], Saskia Bindschedler[1], Pilar Junier[1]

[1] Laboratory of Microbiology, University of Neuchâtel, Neuchâtel
[2] Department of Biology, University of Fribourg, Fribourg

The development of a biological control (biocontrol) system relies on a wide array of experiments in order to find a suitable biocontrol agent against a pathogen of interest. In the case of fungal pathogens, screening and in-vitro experiments often focus on actively growing mycelia. However, this fails to take into account the other developmental stages of relevance for plant infection. Indeed, diseases caused by many fungal pathogens start from the germination of a spore or a sclerotium. Therefore, traditional experiments in biocontrol are not suitable to determine the efficacy of bacteria to control fungal pathogens in which spore or sclerotia are central for pathogenicity. Another drawback of these experiments is that agar makes the recovery and characterization of extracellular metabolites difficult. Therefore, we aim at proposing improved and more targeted methods for in-vitro experiments with spores and sclerotia that can be used in biocontrol, and more widely, in the field of bacterial fungal interactions (BFI). These methods consist of different confrontation assays to assess growth control, recover metabolites or visualise interactions, which can be easily performed in any microbiology laboratory. For this purpose, *Botrytis cinerea*, a phytopathogenic fungus that produces oxalic acid as pathogenicity factor, was used as model to demonstrate the suitability efficiency of the methods we developed. Given that oxalotrophic bacteria have been shown previously to control the growth of *B. cinerea*, the oxalotrophic bacteria *Cupriavidus necator* and *Cupriavidus oxalaticus* were used as model biocontrol agents. Experiments performed with these new methods showed that both bacteria were able to hinder germination of *B. cinerea* spores. The oxalotrophic bacteria also had a negative impact on fungal biomass and fungal metabolites required for a successful infection.

Ribosomal peptides derived from KEX2-processed repeat proteins (KEPs) in fungal physiology, defense and development

Eva Vogt[1], Lukas Sonderegger[1], Marc Flachsmann[1], Antje Dittmann[2], Paolo Nanni[2], Markus Künzler[1]

[1] ETH Zürich, Institute of Microbiology
[2] ETH Zürich, Functional Genomics Center Zürich

Fungal peptides are an important class of bioactive natural products and source of therapeutics. Recently, a novel family of fungal ribosomal peptides, derived from KEX2-processed repeat proteins (KEPs), was defined. KEPs consist of an N-terminal secretion sequence and repeats of short peptide sequences that are separated by dibasic (KR or RR) residues. The dibasic residues are recognized and cleaved by the Golgi endopeptidase KEX2. Additional exopeptidases can further trim the released peptides before they are exocytosed. A well characterized representative of this class of peptides is the α-pheromone of the yeast *Saccharomyces cerevisiae*. Recent biocomputational analysis of 250 fungal genomes suggested that KEPs are widespread and give rise to a broad variety of secretory peptides of yet unknown function. In this project, we are investigating the biosynthesis and function of predicted KEPs and their derived peptides in the mushroom *Coprinopsis cinerea*. Using CRISPR-Cas9 mediated gene knockouts combined with MS-based peptidomics we aim at the identification and structural characterization of the predicted peptides in the supernatant of *C. cinerea* cultures. Additionally, we express the peptide precursors in the yeast *Pichia pastoris* and synthesize the predicted/confirmed mature peptides using a peptide synthesizer. These peptides will be tested for their function in hyphal growth and development as well as fungal defense against bacteria and nematodes. The gained insight will allow to further define this novel class of precursor proteins and might lead to the discovery of novel peptides with interesting bioactivities.

The role of fungal cellulases during plant infection

Francisco Manuel Gámez-Arjona[1], Susanne Dora[1], Clara Sánchez-Rodríguez[1]

[1] ETH Zürich, Institute of Molecular Plant Biology

Climate change is creating new niches for plant pathogens like the ascomycete *Fusarium* spp. Some crops, with an enormous impact on the economy, are affected by this threat. Moreover, their production would also be endangered because some agricultural methods enhance the spread of the disease. To offer solutions to this global problem, we need a piece of reliable knowledge about how the infection is taking place and the players involved. In this sense, the plant cell wall is the first barrier to plant pathogens. Consequently, the fungal cell wall degrading enzymes, like cellulases, have been proposed as critical elements in the initial interaction between plants and pathogens. To elucidate the role of fungal cellulases during infection, we used the model system *Arabidopsis-Fusarium oxysporum.* The strategy was to decrease the levels of fungal cellulases present during the infection. Therefore, we knocked out in *Fusarium* the transcription factor *CLR1*, a putative master transcription regulator involved in the expression of cellulases in ascomycetes. Several cellulases showed a strong downregulation in the *clr1* mutant, and, as a result, the fungus was not capable of degrading cellulose in vitro. Despite this, the *clr1* mutant was more virulent than the wild type version. We carried out *Fusarium* secretome analysis upon infection to explain this phenotype, which showed that multiple virulence factors were significantly enriched in the *clr1* mutant secretome. Additionally, plants infected with wild type fungus displayed higher expression levels of defense related genes than the plants infected with *clr1* mutant. The decrease in plant defense and the increase in fungal virulence factors might explain why the *clr1* mutant is more aggressive, highlighting that cellulose's degradation is not a prerequisite for *Fusarium* infection. Interestingly, we obtained similar results for the pathosystem tomato-*F.oxysporum*, indicating that the regulation of cellulases and virulence factors is well conserved independently of the host.