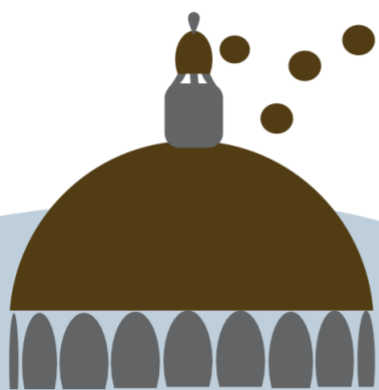


# ABSTRACT BOOK



# ISCLB

**International Symposium on Cereal Leaf Blights,  
5-9 June 2024 in Zurich, Switzerland**

# Abstract Book

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# Keynotes

## Insights from a gall-inducing fungus

Armin Djamei

University of Bonn

Smut fungi are biotrophic specialists in infecting a diverse set of mainly grasses, among them important crops like sorghum, millet, barley and maize. The maize smut fungus *Ustilago maydis* serves as an important model for smuts fungi and induces prominent galls on all aerial parts of its host, reflecting a metabolic and developmental reprogramming of the plant. This massive manipulation of the host is achieved with the help of fungal secreted molecules, so called effectors. In a systematic approach we screened in the past decade hundreds of putative effector proteins to identify their specific place of action and their functions on the plant side. Here I will present our current molecular understanding of the fungal effectome and the biotrophic interaction between the fungus and its host plant maize. Main focus will be given to a group of effectors we identified to promote cell-division, cell expansion and pluripotency by hijacking intrinsic conserved plant signaling pathways.

Keynote

# Mycorrhizas and Microbiome Management for a Sustainable Agriculture

Marcel van der Heijden

University of Zurich & Agroscope

The plant and soil microbiome is highly diverse and comprises over one quarter of Earth's diversity. Yet, how such a diverse and functionally complex microbiome influences ecosystem functioning and sustainability remains unclear. In particular, it is unresolved whether soil microbiome management can help to develop more sustainable agroecosystems. Our research has demonstrated that microbiome management, in particular, inoculation with beneficial arbuscular mycorrhizal fungi (AMF) can promote crop yield and plant health in agricultural fields. We inoculated over 60 agriculture fields and observed that AMF enhanced plant yield in half of the fields with significant growth effects (>15%-40%) in 25% of the fields. Interestingly inoculation success was best predicted by soil microbiome characteristics, specifically pathogen occurrence. Moreover, the application of pesticides in agricultural fields reduced AMF richness and the ability of AMF to acquire nutrients for plants. Finally, by analysing the soil microbiome across >700 sites in Europe we demonstrate the impact of land-use on a wide range of functional groups including plant pathogens. Overall, our work demonstrates that soil microbiome engineering has a large potential to contribute to soil health and sustainable plant production.

# *Special session*

## To Septoria and beyond with some help from my friends

Stephen Goodwin

USDA - Agricultural Research Service

When I began my graduate studies during the fall of 1982 our cutting-edge tool was isozymes to analyze fungal population genetics and required a long period of trial and error to develop a system for each species. No computer programs were available for analyzing population variation so you had to write your own or do the calculations by hand. When I switched to the potato late blight pathogen for a postdoctoral position it turned out that populations of that organism outside of Mexico had very little genetic variation, so DNA fingerprinting was developed to distinguish individuals within clonal populations. I was able to get a fast start to Septoria research by visiting the labs of Bruce McDonald and Gert Kema and by obtaining wheat populations segregating for different resistance genes from other colleagues. Once genomic sequencing technology became widely available we wrote a grant proposal to sequence the genome of the (then called) *Mycosphaerella graminicola*. This was only successful after numerous frantic phone calls while at the Septoria meetings in Tunisia during December of 2003 put us in contact with the Joint Genome Institute (JGI). Annotating the genome was another area of ignorance and inexperience, which we filled by holding annotation jamborees hosted by JGI, and which led to the continuing workshops on Dothideomycetes Genomics. We have gone from a world where there was disagreement about whether resistance to Septoria Tritici Blotch in wheat was quantitative or qualitative, to knowing much about specificity and having several resistance genes cloned. The major problem used to be generating the data and now it is having the data analyzed. Despite amazing progress over the past 40-plus years, there still remains much for new scientists to discover. Maybe during the next 40 years plant pathologists will achieve the goal to put ourselves out of a job!

## The origin and making of IPO323 as a community reference

Gerrit HJ Kema

Wageningen University

The reference strain IPO323 originates from leaves that were sampled by my late colleague Richard Daamen on May 6th in 1981 from the then-important wheat cv. Arminda in a field owned by "farmer Claassen" in southernly West Brabant, The Netherlands. It was isolated on May 7th by our technician Wil Veenbaas at the "Instituut voor Plantenziektenkundig Onderzoek" (Research Institute for Plant Protection, abbreviated as IPO). She was my lab instructor and helped me to get going in Septoria. From there my research program at the IPO started in the late 1980s with a strong focus on the specificity of the host-pathogen interaction. Initially focusing on establishing an international collection of isolates but eventually also studying Dutch isolates to enable field evaluations of these interactions. It was then that IPO323 stood out with a very differential response on wheat cultivars and became the leading isolate in a range of discoveries on sexual development, mapping, transformation and manifold functional analyses, and genome sequencing. As many of these projects were highly collaborative the "Zymo community" was collectively built on the way. Until where you are now, a flourishing community with a great perspective and visibility.



## Molecular interactions between genetics and plant pathology: a fungal point of view

Marc-Henri Lebrun

INRAE

Once upon a time, some geneticists began studying fungal plant pathogens as intriguing subjects for their research. Flor examined the segregation of rust virulence on resistant flax cultivars. Eureka! He discovered that a single gene was involved, allowing him to formulate the simple hypothesis known as the gene-for-gene relationship. This concept is still in use today, despite our current understanding that avirulence genes can interact with each other as enhancers or suppressors. In those early days, fungal genetics primarily relied on classical genetics (segregation in crosses) which was challenging due to asexual nature of many plant pathogenic fungi. This challenge was overcome through worldwide collaborations to identify fertile isolates. Successful crosses were eventually achieved, paving the way for map-based cloning of some of the first avirulence genes. With the advent of international sequencing projects in 2000, fungal genomes were decoded, opening new avenues for fungal molecular genetics and evolutionary studies. These approaches are now facilitated by the ease of obtaining complete genome sequences and transcriptomes. This accumulation of genetic and genomic data has become quite exhaustive, enabling the study of more complex biological processes involving multiple genes, and their interactions, the grail of any geneticist.

## A pleasant journey through the population genetics of fungal plant pathogens

Bruce McDonald

ETH Zurich

Populations of fungal plant pathogens contain enormous reservoirs of genetic diversity that enable rapid evolution when placed under strong directional selection. Population genetic and genomic studies oriented around well defined, naturally infected fields provided many insights into the processes driving pathogen evolution. I'll provide some highly condensed highlights of my professional journey through the population genetics for fungal pathogens, starting with my PhD training at UC Davis, proceeding with the phylogeographical studies initiated at Texas A&M University and completed at ETH Zurich, and ending with the onset of the genomics era for the wheat pathogens *Zymoseptoria tritici* and *Parastagonospora nodorum* and the barley pathogen *Rhynchosporium commune*. The talk will be populated with anecdotes and entertaining photos including only a small fraction of the many wonderful people I was fortunate to meet and work with on this pleasant scientific journey.

## From Snodprot to Snogs

Richard Oliver

Nottingham University

In early 2000, I was asked to start a research group on necrotrophic disease of importance in Australia. One of the two choices was easy because septoria nodorum blotch was both one of the most important necrotrophs and a pathogen with a good (for the time) set of functional genomic tools. SNB technology had been established by Chris Caten and was one of the key pathogens used by the agrochemical industry as a model for fungicide discovery. Together with a select group of colleagues, we set out to uncover genes important in infection, confident that such knowledge would solve the problem. The knowledge did solve a problem but not in the way we had predicted. One key factor was a global collaboration. Currently we can make the claim that SNB is not only one of the best studied plant diseases, it is also one that has generated a large economic return for Australian farmers

# *Lead talks*

## Evolution of the multicopy ToxB gene in *Pyrenophora tritici-repentis*

Reem Aboukhaddour

Agriculture and Agri-Food Canada

Copy-number variation drives evolution in eukaryotes, correlating with increased virulence and adaptability in fungal plant pathogens. In the wheat pathogen *Pyrenophora tritici-repentis* (Ptr), ToxB acts as a major necrotrophic effector, inducing chlorophyll degradation and foliar chlorosis in susceptible wheat genotypes. The ToxB gene varies in copy numbers, ranging from 0 to 10 copies in Ptr. In this presentation, we'll discuss recent research advancements on ToxB and its evolution in tan spot and related species, focusing on its duplication mechanism. Using 24 long-read assemblies (PacBio RS II) with Hi-CANU, we aimed to unravel ToxB's replication mechanism within the Ptr genome. Our results revealed that in isolates with multiple copies, ToxB exists as tandem unidirectional copies, with evidence suggesting Helitron involvement. Furthermore, ToxB resides within a repeat-dense region abundant in transposon activity, with some transposons disrupting the ToxB reading frame. The region containing ToxB is absent in isolates lacking the ToxB gene. The presence of large regions like this suggests potential involvement of unequal crossing-over in ToxB duplication, in addition to Helitron-mediated replication.

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## Accelerating identification of resistance to major wheat fungal threats in Tunisia

Sarra Ben M'Barek

Regional Field Crops Research Center of Beja / Septoria Platform

Wheat is grown in the Maghreb region since antiquity and provides a large part of the nutritional components for food (semolina, flour) and feed (wheat bran, wheat straw). Tunisia is a key durum wheat producer in this region but is also the largest per capita durum wheat consumer in the world. The wheat-based production system in Tunisia is facing challenges to keep up with the growing demand to ensure food security, as supply is largely met by imports currently accounting for over 75% of total needs. In this talk, I will discuss economically important diseases in Tunisia with a focus on Septoria tritici blotch (STB), Tan spot, and Stem rust diseases that are either endemic or re-emerging and raise serious concerns for durum wheat cultivation in Tunisia. Faced with this situation, durum wheat breeding programs launched intensive work in search for novel sources of resistance. Up to date, Septoria resistance genes were characterized mostly on bread wheat while very few were recently identified on durum wheat. CIMMYT in partnership with the Institution of Agricultural Research and Higher Education (IRESA) and the National Institute of Field Crops established a Septoria precision phenotyping platform initially supported by CRP Wheat (CGIAR Research Program on Wheat) promoting the search for resistance to *Zymoseptoria tritici* the causal agent of STB in close cooperation with stakeholders within Tunisia and National Agricultural Research System of North Africa as well as advanced research Institutions and Universities in Italy, France, Canada and United Kingdom. The platform is part of a global network of precision field-based wheat phenotyping, where selected locations at key environments host platforms that generate data on prioritized traits, fostering global germplasm and data exchange. Currently, the platform screens up to 20.000 accessions annually on average at two distinct hot spot locations representing two different climatic zones, complemented with artificial inoculation. Reliable data was obtained on a large number of lines/accessions and good novel resistance sources were identified. The development of molecular markers is under progress to effectively breed for STB resistance from the Mexico-based program. In addition, a collection of USDA Mediterranean durum wheat accessions has been phenotyped for resistance to STB and Tan spot, genotyped using the 90K array, and subsequent genome-wide association analysis (GWAS) was conducted to identify genomic regions associated with resistance to the latter diseases. After the sporadic re-emergence of wheat stem rust disease, caused by *Puccinia graminis* fsp. *tritici* (Pgt), in Tunisia in 2018, disease surveillance, race phenotyping, and genotyping of the local Pgt population have been undertaken over the past cropping seasons. Furthermore, a follow-up study is underway, focusing on whole-genome single nucleotide polymorphisms (SNP) analysis of a global population that comprises predominant races isolated from Tunisia, Spain, Italy, and France. The study aims to explore the genetic diversity and evolutionary patterns of Pgt across different geographical regions. These are some of the important findings that will be raised during my presentation.

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Lead talk

# The cloning of multiple susceptibility genes in wheat reveals diverse host targets hijacked by necrotrophic pathogens

Justin Faris

USDA - Agricultural Research Service

Tan spot and septoria nodorum blotch (SNB) are foliar diseases of wheat caused by the necrotrophic fungal pathogens *Pyrenophora tritici-repentis* (Ptr) and *Parastagonospora nodorum* (Pn). Both pathogens produce necrotrophic effectors (NEs) that are recognized by the products of specific host genes in an 'inverse' gene-for-gene manner leading to programmed cell death and other hallmarks of a classic biotroph defense response, thereby hijacking the plant's innate immune system to cause disease. A primary aim of our research is to identify the wheat genes that recognize Ptr and Pn NEs to gain a better understanding of the molecular mechanisms involved in these pathosystems and to develop functional markers to aid in breeding resistant varieties. Toward that goal, we have cloned and characterized ten wheat genes from these pathosystems that represent five different classes of host genes targeted by these pathogens. The proteins encoded by these genes include both intracellular and membrane spanning proteins, many of which resemble classic biotrophic resistance genes, but some of the genes encode a novel class of protein not previously associated with a pathogen response. In this presentation, I will provide a general overview of the genes cloned in these systems and progress toward their characterization and understanding of their involvement in governing susceptibility to tan spot and SNB.

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# The leaf microbiota of grasses establish interactions with *Zymoseptoria* species beyond antagonism

Victor M. Flores-Nunez

Christian Albrechts Universität zu Kiel

Members of the plant microbiome compete against pathogens and contribute to disease suppression. Moreover, fungal pathogens can antagonize the host microbiome to stimulate disease progression. *Zymoseptoria* species are hemibiotrophic leaf pathogens that specialize in distinct grass species. If different *Zymoseptoria* lineages have encountered differential microbial assemblies during their evolution within their host, we hypothesize that they have specialized to interact with a specific microbiota. We aim to determine the leaf microbiome dynamics of wild grasses and their molecular interactions with host-specific lineages of *Zymoseptoria*. Wild *Aegilops cylindrica*, *Hordeum murinum*, and domesticated *Triticum aestivum* assembled different leaf microbiomes; however infected wild plants experienced fewer changes than wheat during the biotrophic phase of *Zymoseptoria*. Competition assays showed that *Zymoseptoria* inhibits a more diverse subset of wheat-associated bacteria compared to wild host-associated bacteria. However, a higher diversity of bacterial taxa from wild plants exhibited antifungal activity. Remarkably, several bacteria showed enhanced growth when co-cultured with the pathogen. By assessing the interkingdom signals between pathogen and microbiota, we will disentangle the mechanism of their coexistence. Our work suggests that co-adaptation of *Zymoseptoria* and the plant microbiome in the wild leads to a different spectrum of interactions compared to agricultural settings.

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## Life on the wheat leaf: the epiphytic interactions of *Zymoseptoria tritici* with its host

Helen Fones

University of Exeter

Early infection of wheat by *Zymoseptoria tritici*, causal agent of the economically damaging Septoria tritici leaf blotch, is characterised by an 'asymptomatic' phase which lasts around 10 days under optimal conditions. During this time, the fungus shows limited growth and is generally considered to behave as a 'stealth' biotroph, evading plant defences prior to a switch to necrotrophy. We have recently become aware, however, that when *Z. tritici* spores are inoculated onto a leaf, their germination is highly asynchronous and the growth of hyphae often random, with little evidence for directed growth towards the stomata through which they enter the leaf. Thus, the asymptomatic phase of infection comprises both internal biotrophic growth and external, or epiphytic, spores and growing hyphae. The proportion of each varies between isolates; both virulent and avirulent isolates can survive as epiphytes. Moreover, *Z. tritici* does not merely survive on the leaf surface; it can undergo blastosporulation, form biofilms, and, in the case of avirulent isolates, take advantage of wheat defence suppression by virulent isolates to invade the leaf interior. In current research in my lab, we seek to characterise the epiphytic phenotypes of a range of field and reference isolates and determine how this relates to virulence on multiple wheat cultivars. We are also investigating epiphytic nutrient uptake and interaction with other epiphytic microbes.

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Lead talk

## Population biology and comparative genomics of graminicolous *Pyrenophora* fungi

Pierre Gladieux

INRAE

In this presentation, I will provide an overview of our recent work on the population and comparative genomics of graminicolous fungi belonging to the genus *Pyrenophora*. I will describe the population genetic structure in France of the lineage causing the net-form of barley net blotch, *Pyrenophora teres teres*. I will show how this structure allowed us to reveal the existence of two populations of *P. teres teres* at a global level; one associated with winter barley and the other with spring barley. Additionally, I will present the results of our comparative genomic analysis between *Pyrenophora* species associated with various cereals and grasses, and differing in trophic modes (necrotroph vs hemibiotroph) or ecological strategies (specialist vs generalist). Finally, I will discuss the evolutionary history of the ToxB gene, which encodes a host-specific toxin and plays a major role in the pathogenicity of some *Pyrenophora* species.

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## The role of fungal effectors during interactions between wheat and *Zymoseptoria tritici*

Graeme Kettles

University of Birmingham

Likely many pathogens, the wheat-infecting fungus *Zymoseptoria tritici* deploys secreted proteins (effectors) at different phases of its life cycle to manipulate host physiology and aid colonisation. However, in some circumstances effectors can also trigger host immunity through activation of disease resistance proteins. Our group investigates the role of effectors in both of these disease outcomes. During wheat infection, *Z. tritici* undergoes a lengthy symptomless infection phase before the appearance of disease lesions. Although some effectors with immune suppressive activity have been described, we speculated that one reason *Z. tritici* is able to suppress host defence for so long is due to deployment of a large battery of effectors with immune suppressive potential. We used *N. benthamiana* to demonstrate that numerous *Z. tritici* effectors expressed early during wheat infection are also able to suppress cell death and production of reactive oxygen species (ROS) in this model plant. Based on these results, we have investigated the role of a secreted leucine-rich repeat effector (Zt-LRR) which may function through molecular mimicry of host immune receptors. Several *Z. tritici* effectors (Avr proteins) that trigger wheat immunity in a gene-for-gene manner have been described. These genes have been identified through QTL mapping or GWAS approaches that are time-consuming and require considerable technical expertise. We therefore sought to develop a forward genetics approach for Avr gene identification using random mutagenesis. Using the AvrStb6-Stb6 interaction as a test case, we developed a methodology for the high-throughput *in planta* screening of large numbers of UV-mutagenised spores to identify gain-of-virulence (GoV) mutants from an otherwise avirulent genetic background. Whole genome sequencing was used to demonstrate that the majority of recovered GoV mutants harboured mutations in AvrStb6 itself or larger chromosomal deletions involving the AvrStb6 locus. Given these promising results, we are now using this methodology to try and identify currently unknown Avr genes that may be responsible for recent disease resistance breakdowns in the UK.

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Lead talk

# Exploring the genomic landscape of rapid adaptation in the fungal wheat pathogen *Zymoseptoria tritici*

Cécile Lorrain

ETH Zurich

Plant-pathogenic microbes, including the wheat fungal pathogen *Zymoseptoria tritici*, need to adapt to their host environment. The mechanisms underlying host adaptation of *Z. tritici* are still largely unknown. Phenotyping is the main limitation for large-scale GWAS in plant pathogens of several hundreds of strains in multiple hosts. Using natural infection data for GWAS, we reveal from 2 to 13 host-specific candidate per cultivar, including the effector Avr3D1 highlighting the utility of GWAS in unraveling host-specific adaptation mechanisms. We confirm the vast reservoir of genetic variability found in very local populations as well as description of the complex and polygenic genetic basis of *Z. tritici*-wheat interaction, acting as a driving force for evolvability. Growing evidence supports that, transposable elements impact on genome evolution and evolvability. TE activity fosters genetic variability of fungal pathogens directly through insertion within coding or regulatory regions and indirectly by inducing epigenetic modifications. TE-driven modifications can significantly affect fungal pathogenicity by targeting genes involved in virulence. We aim to unravel how the coevolution between transposable elements and fungal genomes has shaped genome evolution, architecture, and adaptation in *Z. tritici*. From host-specific pathogenicity-related genes to TE-driven genome evolution, our research unveils the multifaceted genetic landscape underpinning fungal pathogen adaptation.

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Lead talk

# Sanctuary: A Starship transposon facilitating the movement of the virulence factor ToxA in fungal wheat pathogens

Megan McDonald

University of Birmingham

The 14 kbp ToxhAT transposon has been shown to be moving the necrotrophic effector, ToxA, horizontally between fungal species that infect wheat; *Parastagonospora nodorum*, *Pyrenophora tritici-repentis*, and *Bipolaris sorokiniana*. Here we confirm the movement of ToxhAT using long-read de novo assembly of eight novel and one previously published *B. sorokiniana* isolates including the identification of "TA" target site duplications. These assemblies revealed that ToxhAT is a passenger embedded in a much larger, 170–196 kbp transposon. This element, termed Sanctuary, belongs to the newly described Starship transposon superfamily. We also show that ToxhAT has been independently captured by two different Starships, Sanctuary and Horizon which share little to no sequenced identity, outside of ToxhAT. This classification makes Horizon and Sanctuary part of a growing number of Starships involved in the horizontal gene transfer of adaptive genetic material between fungal species.

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## The diversity of genes involved in wheat resistance to *Septoria tritici* blotch

Cyrille Saintenac

INRAE

*Septoria tritici* blotch (STB), caused by the fungal pathogen *Zymoseptoria tritici*, poses a significant threat to wheat production, particularly in Europe. Understanding the diversity of plant resistance mechanisms is crucial for developing effective management strategies against this devastating disease. Various forms of resistance have been referenced against this pathogen, ranging from non-host resistance to the major Stb genes. Since the first mapping efforts in the early 2000s, 23 Stb genes originated from diverse wheat genetic materials have now been reported on the wheat genome, along with several QTLs, although the distinction between quantitative resistance and the Stb genes remains somewhat debatable. Recently, the first three major Stb genes were identified, encoding receptor-like protein kinases from three different sub-families. Efforts to clone other major Stb genes to further explore the diversity of genes involved and their resistance mechanisms have been initiated. The latest updates on the identification of these resistance genes will be presented. Additionally, quantitative cytological analyses of *Z. tritici* interactions with plant species carrying various forms of resistance including major Stb genes have been conducted to compare these resistances. These findings suggest the presence of a major common resistance mechanism across the diverse forms of resistance against *Z. tritici*.

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## *Zymoseptoria tritici* effectors: Understanding their regulation and wheat targets

Andrea Sanchez Vallet

Centro de Biotecnología y Genómica de Plantas (UPM)

*Zymoseptoria tritici*, the causal agent of septoria tritici blotch, is one of the most damaging wheat pathogens, causing significant economic losses. Understanding the molecular mechanisms underlying resistance towards this pathogen is critical for controlling the disease. *Z. tritici* employs effectors in order to colonize the host and culminate its life cycle. Although effectors are usually beneficial for the pathogen, wheat has evolved mechanisms to specifically recognize certain effectors and to trigger immune responses that hinder the progression of the pathogen. In addition to host resistance, the outcome of *Z. tritici* infections depends on its capacity to compete with other microorganisms that grow as endophytes in wheat leaves. Here, I will present our latest results regarding the contribution of effectors in triggering host resistance and in interacting with fungal endophytes, and the role of effector gene regulation in host evasion.

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Lead talk

# From population dynamics to the prediction and management of fungicide resistance: the case of *Zymoseptoria tritici*

Anne-Sophie Walker

INRAE BIOGER

Fungicide resistance is a textbook example of adaptation, which is all the more facilitated in the wheat pathogen *Zymoseptoria tritici* since this species exhibits high evolvability and disease control still mostly relies on the application of chemicals. Understanding the dynamics of resistance at large geographical scales, as well as trying to predict resistance determinants and population evolution become critical to define more sustainable containment strategies. Here, I will discuss how integrating such information might be useful to design smart anti-resistance strategies. We combine multiple approaches such as phenotypic trait variation, mathematical modeling and experimental evolution, as deployed in the work of four PhD students (Maxime Garnault, Guillaume Fouché, Agathe Ballu, Simon Patry-Leclaire). I will finally debate on why combining control measures is the most promising option to delay the overall adaption of pathogens.

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# *Regular talks*

# Image-based symptom tracking to decompose quantitative resistance in the field

Jonas Anderegg

ETH Zurich

Quantitative disease resistance (QR) is a complex, dynamic trait that is most reliably quantified in field-grown crops. Traditional disease assessments offer limited potential to disentangle the contributions of different components to overall QR. Yet, a better functional understanding of QR could greatly support a more targeted, knowledge-based selection for QR and improve predictions of seasonal epidemics. We have developed a simple, affordable, and easy-to-operate imaging procedure for in-field acquisition of wheat leaf image sequences. The development of *Septoria tritici* blotch and leaf rusts was monitored over time via robust methods for symptom detection and segmentation, image registration, symptom tracking, and leaf- and symptom characterization. Our pilot data enabled the monitoring of 13,538 necrotic lesions on 300 leaves, which provided 72,005 lesion property measurements. Contrasting patterns in lesion numbers and lesion expansion dynamics were observed across wheat cultivars. The number of separate infection events and average lesion size contributed to varying degrees to overall disease intensity, possibly indicating distinct mechanisms of QR. The proposed methodology enables rapid, non-destructive, and reproducible measurement of several key epidemiological parameters under natural field conditions. Such data can support decomposition and functional understanding of QR as well as the parameterization, fine-tuning, and validation of epidemiological models.

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Regular talk

## Exploring transposable element-mediated adaptive trait evolution using a large fungal pathogen genome panel

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Transposable elements (TEs) are implicated in adaptation in several species, but identifying adaptive TE insertions is challenging due to difficulties in confidently calling TE insertions from short-read data and a lack of phenotypic information. Examples of TE-mediated adaptation in *Zymoseptoria tritici* exist, but the role of TEs in the species' global spread remains unknown. Rapid adaptation to new environments and increases in TE activity present an opportunity to systematically investigate the importance of TEs as a source of adaptive variation. Leveraging 2,229 genomes sampled across the globe, we explore the dynamics of TEs under positive selection and their involvement in adaptive trait variation. We find significant TE expansions associated with specific populations, with large numbers of population-specific TE loci rising to high frequencies. We assess candidate polymorphic TEs for signatures of selection to determine their importance in the global spread of *Z. tritici*.

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## Exploring the role in virulence of a *Zymoseptoria tritici* candidate effector during wheat infection

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Plant-associated microbes secrete molecules known as effectors to promote defense manipulation during host colonization. This research focuses on *Zymoseptoria tritici*, a devastating fungal pathogen in wheat, causing the disease Septoria tritici blotch disease (STB). Here, we investigate the functional role of the protein effector candidate Zt190, hypothesized to contribute to defense manipulation during wheat infection. Comparative transcriptomic analyses showed that Zt190 is highly expressed during early host colonization in multiple isolates of *Z. tritici*. Furthermore, proteome analyses showed that Zt190 is as one of the most abundant *Z. tritici* proteins in the wheat apoplast during infection. Transient expression of Zt190 led to suppression of plant immune responses in *Nicotiana benthamiana*. In this system, several putative plant protein interaction partners of Zt190 were also identified through immunoprecipitation coupled to mass-spectrometry based proteomics (IP/MS), among them a pathogenesis-related (PR) protein 15/16 (PR-15/16). Furthermore, infection of a susceptible wheat cultivar with a *Z. tritici* mutant lacking Zt190 showed a reduction in disease symptoms compared to wheat infected with the wild type strain, suggesting this putative effector could be relevant during plant infection. Ongoing research involves performing additional assays to confirm the role of Zt190 in *Z. tritici* virulence.

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## Improved field and environmental diagnostics for sustainable pest management

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Fungal plant pathogens are among the most significant threats to global food security. Currently, disease control relies largely on fungicide applications, but an increased number of resistance outbreaks has been recently reported. This situation, coupled with the societal and political decisions to limit the use of phytosanitary products, requires new and more sophisticated strategies for pest management. Here we mainly focus on *Zymoseptoria tritici*, the causal agent of Septoria leaf blotch of wheat. We screened a collection of a few thousand *Z. tritici* strains for fungicide sensitivity and their related genetic determinants. The results were combined to calibrate, for the most relevant fungicides, population adaptation indexes. The profiling of pre-selected populations either from environmental or field diseased material were related with the field efficacy of spray applications. This approach allows the development of new strategies for maximizing disease control and resistance management. In addition, fungal metagenomic analyses of European environmental samples highlighted, among the others, some unexpected results such as a relevant detection of *Pyrenophora tritici-repentis*. A network of spore traps is proposed as a surveillance tool for precision agriculture. The presented approach will result in the generation of a large set of data, which can be used to offer relevant information for modelling and supporting the definition of a sustainable disease control management.

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# Leveraging Neural Networks Trained on Genomic Sequences for Insights into Fungal Pathogen Genomics

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While AI has found application across diverse domains, its potential in uncovering novel insights in the realm of plant pathology is limited mostly to computer vision models for symptom recognition. Unlike traditional algorithms, often relying on hardcoded assumptions, AI algorithms can automatically discern patterns in data, potentially revealing undiscovered commonalities. Motivated by the success of natural language models in both general applications (e.g., ChatGPT) and biology (e.g., AlphaFold), we aim to harness these advancements for two primary objectives. Firstly, we aim to enhance gene annotation in the absence of transcriptomic data, in particular on pathogenicity-related genes such as effectors. Secondly, we seek to leverage the model's comprehension of genomic syntax to unveil new insights into the DNA language across different fungal groups. Our results show that, without being trained explicitly on this, a genomic AI model can predict syntactic roles of genomic positions, including the identification of start codons, or introns across phylogenetic groups. Our ongoing efforts involve using explainable AI to elucidate the signals recognized by the model within sequences, thus advancing our understanding of genetic syntax variations among fungal groups. This research underscores the potential of AI-driven approaches in deciphering complex genomic landscapes and offers promising avenues for future exploration in pathogen genomics.

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# Mechanisms of infection and response of the fungal wheat pathogen *Zymoseptoria tritici* during compatible, incompatible and non-host interactions

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We investigated the transcriptome activation in *Z. tritici* during infection of susceptible (Taichung 29) and resistant (Veranopolis and Israel 493) wheat cultivars, plus non-host barley at 1, 3, 6, 10, 17 and 23 days post-inoculation (DPI). We observed dramatic differences in pathogen gene expression at 10 DPI in compatible versus incompatible interactions, correlating with the initiation of the necrotrophic lifestyle. The largest differences in pathogen gene expression occurred at 3 DPI in both compatible and incompatible interactions compared to the non-host. We selected thirty-one putative effectors expressed at 1 and 3 DPI in the compatible interaction. Subsequent subcellular localization studies in *N. benthamiana* revealed two candidate effectors that localize to mobile cytosolic bodies, suggesting involvement in intracellular signaling or host gene regulation. Mycgr3109710, which localized to cytosolic bodies, belongs to the non-plant PR-1-like protein family implicated in virulence in other pathogens. We found that Mycgr3109710 contains three CAP signature motifs and a conserved CNY motif, which is present in PR-1-L proteins with confirmed virulence activity in other fungal pathogens. We are studying the role of Mycgr3109710 in cell death induction and ROS attenuation. Also, we are investigating the phylogeny and evolution of PR-1-L proteins in *Z. tritici* using 750 PR-1-L proteins obtained from a BLASTP search of Mycgr3109710 sequence against the Uniref50 database.

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# TE-driven rapid adaptive evolution of the fungal pathogen *Zymoseptoria tritici* against Stb16 resistance gene

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Understanding adaptive evolution in response to biotic stressors in plant pathogen remains a challenge for Agriculture and Health. Here, we study adaptive evolution of the fungal ascomycete *Zymoseptoria tritici*, one of the major pathogens threatening wheat yield worldwide. A recent breakdown of the wheat resistance mediated by the Stb16q gene presents an opportunity to study contemporary evolution of the pathogen's adaptive response. A few years after its massive introduction into wheat elite varieties, this gene no longer confers resistance to an increasing number of *Z. tritici* isolates. This study delves into the molecular mechanisms underlying this adaptation. We unveiled the genetic basis of the new *Z. tritici* virulence on Stb16q, by combining QTL mapping, genome-wide association studies and long read sequencing. Employing *Agrobacterium tumefaciens*-mediated transformation, we validated the role of an effector and demonstrated that a TE insertion within the coding region of the gene otherwise recognized by the host, is the causal variant associated with high level of virulence. This work highlights the primary role of transposable elements in fungal pathogen genome evolution by playing a key part in the evolutionary arms race against plant immunity, resulting in a fitness advantage for the pathogen.

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## Unraveling the importance of *Pyrenophora tritici-repentis* necrotrophic effectors behind tan spot epidemics in the Nordics

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*Pyrenophora tritici-repentis* (Ptr) is a necrotrophic fungal pathogen that causes tan spot of wheat, one of the most prevalent diseases of spring wheat in Nordics. Three necrotrophic effectors of Ptr are currently known: ToxA, ToxB and ToxC. Here we tested sensitivities of 199 Nordic spring wheat cultivars and breeding lines to purified effector proteins ToxA and ToxB and compared the results with tan spot severity data in field trials over two years. We also screened a total of 220 recent and historical Ptr isolates for the presence of ToxA and ToxB genes, and further examined the virulence of a subset of 25 isolates with a wheat differential set to better understand the pathogenicity of Nordic Ptr population. 47 % of the spring wheat genotypes were sensitive to ToxA, and 24 % to ToxB. Despite the presence of ToxB sensitivity in wheat, none of the Ptr isolates carried ToxB gene, whereas ToxA was present in 65 % of the isolates. Virulence testing indicated the presence of ToxC and some unknown factors driving symptom development. Analysis of field data indicated that ToxA sensitivity could explain only a small share of observed phenotypic variation, and the effect of ToxB sensitivity was insignificant. Both ToxA and ToxB have previously been shown to be important factors facilitating disease susceptibility in wheat genotypes carrying sensitivity. Our results suggest factors other than ToxA and ToxB playing an important role in tan spot disease development in Nordic countries.

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## Exploration of synthetic wheats for resistance to Septoria leaf blotch disease

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SLB is one of the most damaging foliar diseases of wheat caused by the fungal pathogen *Zymoseptoria tritici*. High levels of genetic diversity and a very large population size drive evolution of resistance to fungicides and breakdown of host disease resistance. Breeding wheats with high level of SLB resistance is a priority. NIAB have developed a series of synthetic hexaploid wheat (SHW) lines from crosses between different durum wheat and wild goat grass accessions, and these and derived mapping populations are being screened for resistance to SLB under controlled and field conditions. Our results to date revealed high level of resistance among SHWs under controlled and field conditions. Further, we identified several genome regions carrying resistance to multiple fungal isolates. These will be further defined, and the resistance mechanisms explored. This will provide a novel genetic armoury in the breeding of multi-isolate resistant wheats for sustainable SLB management.

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## Mediterranean durum wheat: A genetically diverse panel harboring valuable sources of resistance to tan spot disease

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The production of durum wheat is threatened by biotic stresses including tan spot (TS), a foliar disease caused by *Pyrenophora tritici-repentis*. Little is known regarding TS resistance in durum compared with common wheat. In this study, we evaluated the population structure, genetic diversity, and resistance to TS at the adult plant and seedling stages of a collection of durum landraces, elite cultivars and breeding material from 11 countries in the Mediterranean Rim. Two-hundred fifty-eight accessions were genotyped using DArTseq technology and genome-wide association analysis (GWAS) was conducted to identify markers associated with TS resistance. Discriminant analysis of principal components (DAPC) using single nucleotide polymorphism (SNP) markers indicated seven populations, while STRUCTURE analysis identified two populations. The variation observed among populations was 26.0% of the total, whereas 61.3% and 12.8% of the variation was attributed to individuals within populations and differences between individuals, respectively. The broad sense heritability estimate for TS resistance was equal to 0.60. GWAS identified 29 SNPs associated with TS resistance that explained 4.4% to 13.1% of the phenotypic variation, and which were located on all chromosomes with the exceptions of 4A, 5A and 5B. The results indicate that chromosomes 2B, 7A and 7B play an important role in TS resistance, and that the identified resistant genotypes could be incorporated in breeding efforts.

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## Killer proteins 4 and 6 from the fungal wheat pathogen *Zymoseptoria tritici* are toxic to fungi and structurally related to effector families

Marc-Henri Lebrun

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Effectors from fungal plant pathogens are challenging to classify due to their low sequence identity. However, understanding their structure allows for their classification into families. The structures of two putative effectors from the wheat fungal pathogen *Zymoseptoria tritici* were determined using X-ray crystallography. Zt-Mycgr3-91409 is a candidate MAX effector, while Zt-NIP1 induces wheat leaf necrosis. Surprisingly, these two proteins were structurally related to KP6a and KP4 killer toxins, respectively, encoded by UmV dsRNA viruses infecting the corn smut *Ustilago maydis*. Zt-Mycgr3-91409 and Zt-NIP1 were renamed Zt-KP6-1 and Zt-KP4-1. Homologs of Zt-KP6-1 and Zt-KP4-1 were identified in *Zymoseptoria* species. Fungal proteins structurally related to Zt-KP6-1 and Zt-KP4-1 were identified using a novel pipeline based on Foldseek and HMM searches. These Zt-KP6-1 and Zt-KP4-1 like proteins were widely distributed across fungi. The biological activity of Zt-KP6-1 and Zt-KP4-1 was assessed on plants and fungi. Infiltration of Zt-KP6-1 and Zt-KP4-1 into wheat leaves did not induce visible symptoms. Zt-KP6-1 completely inhibited the growth of *Botrytis cinerea*, while it was less active on *Z. tritici*. Zt-KP4-1 was toxic to both *B. cinerea* and *Z. tritici*, demonstrating that these effectors are toxic to fungi. This study showed the importance of structure prediction in understanding the function of fungal effectors.

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## Toward understanding of the molecular basis of the Ptr ToxC production in wheat tan spot pathogen *Pyrenophora tritici-repentis*

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*Pyrenophora tritici-repentis* is known to produce necrotrophic effectors (NEs) to cause tan spot in wheat. One of them is Ptr ToxC which was initially characterized as a secondary metabolite, but the molecular basis of its production remains largely unknown. We recently mapped and cloned a fungal gene (ToxC1) that is required but not sufficient for the Ptr ToxC production. To identify additional genes, we performed a transcriptomics study on a Ptr ToxC sensitive line using a race 1 isolate and its ToxC1 mutant. Three genes closed to ToxC1 were identified as candidates due to their similar expression pattern as ToxC1. Among them, one is a homolog of ToxC1, and the other two encode a hydroxylase and a secreted small protein. All three genes are shown to be also required. However, a polyketide synthase gene adjacent to the region was shown not to be involved in the Ptr ToxC production. This work provides a better understanding of the biosynthetic pathway of Ptr ToxC.

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## Ducks in a row: multiple effectors of *Pyrenophora teres f. teres* target a single host susceptibility locus in barley

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*Pyrenophora teres f. teres* causes net form net blotch in barley. A biparental population of isolates 15A and 6A was used to identify two quantitative trait loci (QTL), VR1 and VR2, associated with virulence on Rika barley. Two effector gene candidates, VR1 and VR2, were selected for functional analysis. Both genes were universally present in a global *P. teres f. teres* collection with VR1 encoding for a secreted peptidase and VR2 for a secreted hypothetical protein. Single and double VR1 and VR2 gene disruptions in isolate 6A caused a significant reduction in virulence on Rika. Gene-edited strains acquiring the virulent 6A VR1 and VR2 alleles became more virulent on Rika. Inoculation of the gene-edited strains onto the Rika × Kombar (RK) host population showed that both VR1 and VR2 targeted the susceptibility locus Spt1 in Rika. Therefore, VR1 and VR2 act as effector genes and independently contribute to virulence by targeting the susceptibility locus Spt1. Interestingly, a significant QTL targeting Spt1 was still observed when the 6A VR1 and VR2 double mutant (6A $\Delta$ vr1 $\Delta$ vr2) was inoculated onto the RK population. Therefore, since 6A $\Delta$ vr1 $\Delta$ vr2 was still virulent and this virulence targeted Spt1, additional effectors are likely produced by 6A and may be harboured in the VR1 or VR2 QTL.

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## The specialization of *Zymoseptoria tritici* on durum wheat

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*Zymoseptoria tritici* is a fungal pathogen causing the Septoria tritici blotch disease on different host species, including bread wheat (*Triticum aestivum*) and durum wheat (*T. turgidum* ssp. *durum*). *Z. tritici* isolates are usually strongly specialized in the infection of one host species, although some studies reported isolates capable of infecting multiple hosts. To gain insights into the genetics of the interaction between durum wheat and *Z. tritici*, we established a large collection of *Z. tritici* isolates collected on durum wheat varieties grown in France, Italia, Spain and Tunisia. We sequenced the whole genome of 120 of these isolates to study the distribution of genetic diversity between the different production areas of durum wheat and to replace this diversity in the global Euro-Mediterranean *Z. tritici* population. Then, we evaluated the capacity of these 120 isolates to cause symptoms on a differential series of four bread wheat and 16 durum wheat varieties carrying different resistance genes in order to reveal their level of specialization at the species and variety levels. Finally, we gained insights into the genetic determinants involved in this specialization by genetically mapping resistances in two bread wheat and six durum wheat biparental populations (issued from crosses with varieties from the aforementioned differential series) as well as pathogenicity genes of *Z. tritici* on these same resistant varieties.

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# Local adaptation to temperature in a genetically diverse world-wide collection of a major plant pathogen

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Environmental factors, such as temperature, significantly affect the growth and fitness of plant pathogens throughout their life cycles. Adaptation to new temperature regimes is one of the key challenges that a plant pathogen might face during range expansion. The wheat pathogen *Zymoseptoria tritici* is a compelling model to study temperature adaptation due to its wide distribution requiring its survival in several climates. While previous studies have explored the response of this pathogen to temperature, evidence for adaptation remains limited, mostly focusing on European populations. In this study, we aimed to evaluate the local adaptation of *Z. tritici* to temperature by using the response of 411 world-wide distributed isolates to different temperatures using in-vitro phenotyping. Our investigation reveals substantial variability in thermal performance and high genomic diversity at both individual and population levels. Leveraging this diverse global collection, we identified novel genetic variants involved in adaptation to temperature through genome wide association analyses (GWAS). By providing insights into the mechanism governing local adaptation in *Z. tritici*, our research enhances our understanding of how pathogens can access ecological niches that were not previously favorable.

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## Using *Nicotiana benthamiana* to understand non-host resistance against *Zymoseptoria tritici*

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The *Z. tritici* effectors Zt9, Zt11 and Zt12 are recognised in the non-host plant *N. benthamiana* and induce BAK-1 dependent cell death. Using a forward genetic screen, we aim to identify the immune receptors controlling recognition of these effectors in *N. benthamiana*. To induce mutations, *N. benthamiana* seeds were treated with 0.4% Ethyl methanesulfonate (EMS). Subsequently, we cultivated and selfed 1500 mutagenised plants to establish an M2 mutant population. Screenings were performed to evaluate absence of the cell death (CD) phenotype in response to three *Z. tritici* effectors using *Agrobacterium*-mediated transient expression. Of the 750 lines screened, six exhibited a lack of CD response to one or more of the three *Z. tritici* effectors. Underlying mutations are currently being identified using bulked segregant analysis. In addition, we are utilising TurboID proximity labeling to identify interacting proteins of these effectors. Our most recent findings will be presented.

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## The status of net form net blotch of barley in Western Australia using high-resolution phenotyping and multi-omic analyses

Fatima Naim

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Net blotch is a damaging barley disease caused by the necrotrophic fungal pathogen *Pyrenophora teres*. It occurs in two phenotypically distinct forms: net form net blotch caused by *Pyrenophora teres* f. *teres* (Ptt), and spot form net blotch caused by *Pyrenophora teres* f. *maculata* (Ptm). The recent emergence of fungicide resistant and increasingly virulent pathotypes and lack of resistant barley germplasm pose a serious threat on our barley production. To understand the mechanisms of pathogenicity and differences in virulence, and differential phenotypes, we have applied spatially resolved high-resolution microscopy and multi-omic techniques to barley leaves infected with Ptt. This included a combination of synchrotron and confocal microscopy, long-read genome assemblies of the pathogen strains, and gene expression using short and long read sequencing from targeted tissue sampling. I will present our recent findings in disentangling this complex interaction paving the way to better management of the disease.

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## Exploring the role of the *Parastagonospora nodorum* necrotrophic effector, SnTox267, during *in planta* colonization

Ashley Nelson

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*Parastagonospora nodorum* is a necrotrophic fungal pathogen that causes septoria nodorum blotch (SNB) on wheat. There have been five necrotrophic effectors identified, cloned, and found to contribute to *P. nodorum* virulence. The necrotrophic effector SnTox267 was originally thought to be three individual effectors (i.e., SnTox2, SnTox6 and SnTox7) that targeted wheat susceptibility genes Snn2, Snn6 and Snn7, respectively. However, recently it was discovered that SnTox267 is a single proteinaceous necrotrophic effector, targeting at least two separate pathways in wheat. Here we characterize in-*planta* colonization by comparing a wild type *P. nodorum* isolate, Sn4 with its SnTox267 disruption strain (Sn4 $\Delta$ tox267) on wheat lines BG284 (SnTox267 insensitive), BG223 (SnTox267 sensitive) and Grandin (SnTox267 sensitive). Samples were stained using fluorescent dye, analyzed using confocal microscopy, and quantified using machine learning assisted volume analysis. In addition to this, reactive oxygen species production was measured using 3-3'-Diaminobenzene (DAB) staining, which is oxidized by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and nitroblue tetrazolium (NBT) staining, which is oxidized by superoxide anions (O<sub>2</sub><sup>-</sup>). Using these, we can begin to characterize the temporal and spatial role of SnTox267 *in planta*.

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## The complex genetic landscape of fungicide resistance evolution in *Zymoseptoria tritici*

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Over the past three decades, the extensive use of target-site fungicides, such as demethylation inhibitors (DMIs) and succinate dehydrogenases inhibitor (SDHIs), in European agricultural fields has turned the continent into an ideal geographic range for identifying the emergence of specific resistance mutations over time and space. Structural alterations in the target protein are the most common mechanism leading to insensitivity to DMIs as SDHIs. However, there is compelling evidence suggesting that additional genetic loci also contribute to overall resistance. In this study, our objective is to unravel the evolutionary trajectories driving the genetic architecture of fungicide resistance in *Zymoseptoria tritici*, the major wheat pathogen in Europe. To achieve this, we utilized a panel consisting of 1420 whole-genome sequenced isolates from 27 European countries, spanning a period of 15 years. By employing multiple phenotyping and genotyping techniques, we conducted genome-wide association mapping to identify the genetic factors associated with resistance. As a result, we discovered numerous previously unknown loci that contribute to DMI resistance, in addition to mutations in the gene responsible for encoding the DMI target protein. Remarkably, despite most fungicides are targeting specific proteins, our findings reveals multiple loci linked to resistance adaptation.

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# Diversification, loss, and virulence gains of AvrStb6 during continental expansion of *Zymoseptoria tritici*

Margarida Sampaio

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Interactions between plant pathogens and their hosts are highly dynamic and mainly driven by pathogen effectors (Avr) and plant resistance (R) genes. AvrStb6 is the best studied *Zymoseptoria tritici* effector and it is recognized by Stb6, a widely deployed wheat R gene. AvrStb6 is known to be highly polymorphic across the world, however, how the gene evolved in response to strong selection pressure by the host remains poorly understood. Here, we analyzed a global thousand-genome panel to assess the diversification of AvrStb6 and the genomic context. We detected 59 AvrStb6 protein isoforms in the global collection with the most frequently detected isoform in Europe showing the strongest divergence from the *Z. pseudotritici* sister species homolog. We found indications that AvrStb6 diversified most strongly in regions with supposedly higher Stb6 deployment rates. AvrStb6 showed also remarkably diversification in transposable element associations near the coding sequence, and we detected complete gene loss in ~3% of all surveyed isolates. Finally, we used genome-wide association mapping data to predict virulence profiles for isoforms present across continents. We found that populations showed marked increases in predicted virulence in Europe and subsequently colonized continents. In conclusion, we show how a rapidly diversifying effector loci can undergo large-scale sequence changes concomitant with gains in virulence on cognizant cultivars.

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# Wheat blast - an expanding disease threatening global wheat production

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Wheat blast is caused by *Magnaporthe oryzae* pathotype *Triticum* (MoT), which was initially observed in South America, this disease remained confined there until a significant outbreak in Bangladesh in 2016, indicating its spread from South America to South Asia. In 2018, the presence of wheat blast in Zambia marked its first reported occurrence on the African continent. Prediction models, based on agro-climatic factors, have highlighted extensive regions with warm and humid conditions as being at risk, a situation that could worsen with climate change and the pathogen's potential adaptation to cooler and dryer climates. Strategies to cope with the disease include wheat holiday, seed treatments, fungicide application, to host resistance. However, breeding efforts face hurdles due to the scarcity of resistant sources and rapid evolution of MoT. Seed treatments are effective against seedling infections but are ineffective against spike infections. While fungicides can offer some preventive benefits, their efficacy is diminishing due to the fast emergence of fungicide resistance of MoT. Adjusting sowing time is the most commonly implemented cultural strategy in areas affected by wheat blast. Managing wheat blast presents a complex challenge that necessitates a comprehensive, interdisciplinary approach and international cooperation to minimize its detrimental impacts.

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## Rapid mitochondrial genome structure evolution in *Zymoseptoria tritici* and beyond

Ivan Skakov

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Mitochondria play crucial roles in eukaryotic cells, serving as hubs for ATP production and encoding their own genomes. Fungal mitogenomes exhibit high diversity in both size and composition, yet this diversity has primarily been characterized at the genus or higher levels. In our study, we focused on analyzing mitogenome variation generated over short evolutionary time within species, by examining thousands of strains of the major wheat pathogen *Zymoseptoria tritici*. We uncovered incongruences between mitochondrial and nuclear population structures, as mitogenome genetic clusters did not correspond to nuclear genetic clusters. To reveal structural variation underpinning mitogenome diversity, we assembled ~2000 mitochondrial genomes based on long-reading and short-read sequencing. We found four primary haplotypes distinguished mainly by two large (up to ~10% of the entire mitogenome) insertion/deletions. Notably, a small subset of genomes shared an additional sequence encoding a selfish genetic element identified as a GIY-YIG homing endonuclease (HE). Comparative analyses of mitogenomes from closely related *Zymoseptoria* species revealed substantial variability at the genus level and revealed the closest orthologs of the HE gene. This suggests that a deletion affecting the GIY-YIG HE occurred early in the evolution of *Z. tritici*. Our findings shed light on the extensive variability of mitochondrial genomes over short evolutionary time spans.

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# Impact of sexual reproduction on adaptive dynamics of *Z. tritici* populations driven by R-AVR interaction

Frédéric Suffert

INRAE BIOGER

Little is actually known about the impact of host immunity on sexual reproduction in *Zymoseptoria tritici* but also reciprocally about the impact of sexual reproduction on epidemiological processes and adaptive dynamics of pathogen populations driven by R-AVR interactions. An experiment investigating different scenarios of *Z. tritici* crosses on wheat according to the virulence or avirulence status of the parents against a *Stb* gene showed that parents did not require preliminary symptomatic asexual infection to mate. This change in the population genetics baseline of *Z. tritici* deserves particular attention in heterogeneous wheat covers (cultivar mixtures or landraces), where interactions between virulent and avirulent strains are favoured. Depending on the conditions, crosses could either limit resistance breakdown by maintaining the frequency of virulent strains below a certain threshold, or conversely, increase it, even promoting the emergence of multi-virulent strains.

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## The wheat pathogen, *Zymoseptoria tritici*, expresses effectors that bind lipid transfer proteins (LTPs; PR-14)

Eli Thynne

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Before inducing necrotic disease symptoms, the wheat pathogen, *Zymoseptoria tritici*, undergoes an asymptomatic growth phase of almost two weeks. During this time, the pathogen grows throughout the host's apoplast, evading immune responses. However, the wheat immune system is not dormant, and numerous immune-related genes are highly expressed while *Z. tritici* infects. Among these are lipid transfer proteins (LTPs; PR-14). LTPs are highly abundant apoplastic proteins during *Z. tritici*'s infection, and can function as antifungals. They have also been reported as immune-signaling molecules, associating with hormones, and another PR protein, PR-1, to enhance resistance. Despite this, *Z. tritici* can tolerate the presence of LTPs during infection. We have identified a class of effectors expressed by *Z. tritici* that can interact directly with LTPs. We are exploring if these effectors can inhibit LTPs' antifungal activity and their interaction with PR-1. Transient expression of these effectors in the non-host, *Nicotiana benthamiana*, can lead to cell-death. Interestingly, *N. benthamiana* has a receptor-like protein (RLP), that interacts with LTPs coplexed with jasmonic acid. Silencing of this RLP (or the RLP co-receptor SOBIR1), inhibits LTP-binding effector-induced cell-death, indicating that it is responsible for this non-host effector recognition. This RLP could potentially represent a source of disease resistance to fungal pathogens that express this class of LTP-binding effectors.

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## Enhancing STB Resistance in Australian Wheat Breeding through Multi-Stage Resistance loci

Nannan Yang

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*Zymoseptoria tritici*, commonly known as Septoria tritici blotch (STB), poses a significant threat to wheat production in Australia. Over the past decade, our research has suggested the presence of Quantitative Trait Loci (QTL) that provide resistance to the development of disease at multiple growth stages in plants. Multi-stage resistance (MSR) can be considered different and distinct from Adult Plant Resistance (APR), as the resistance is continuously expressed through progressive crop development stages from seedlings to grain-filling. MSR loci that can provide resistance, such as seedling-stage resistance that reduces early levels of infection, as well as APR-type resistance that protects green leaf area at later growth stages, should be attractive breeding targets for cultivar development. Evidence on QTLs/genes involved in MSR will be presented. Additionally, the current status and progress on STB resistance breeding in Australia will be discussed.

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# Towards high throughput in-field detection and quantification of wheat foliar diseases with deep learning

Radek Zenkl

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Reliable, quantitative information on the presence and severity of crop diseases is critical for site-specific crop management and resistance breeding. Successful analysis of leaves under naturally variable lighting, presenting multiple disorders, and across phenological stages is a critical step towards high-throughput disease assessments directly in the field. Here, we demonstrate the capability of deep learning based keypoint detection for STB pycnidia and rust pustules combined with semantic segmentation for leaves, leaf necrosis and insect damage to reliably detect and quantify the presence of Septoria Tritici Blotch, leaf rusts, and insect damage under such conditions. In addition we present the Eschikon Wheat Foliar Disease (EFD) dataset with 418 high resolution images with polygon annotations of leaves, leaf necrosis and insect damage and point annotations of STB pycnidia and rust pustules. With our dataset a solid benchmark is established, but the potential of the proposed method remains under-exploited as significantly more data containing more diverse symptoms can be utilized to further improve the performance and introduce new disorders. Finally, we demonstrated the robustness of the approach by evaluating images of an unstructured canopy. This underlines the potential for extending this work to make more efficient in-field analysis without the need to detach leaves and thus moving towards automated in-field assessment of foliar diseases.

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# Posters

# A whole-genome SNP analysis for genetic diversity characterization of the re-emerged *Puccinia graminis* f. sp. *tritici* population in Tunisia

Wided Abdedayem

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Wheat stem rust caused by *Puccinia graminis* f.sp. *tritici* (Pgt) is one of the most destructive wheat diseases worldwide. The disease re-emergence has been reported since the Sicilian outbreak in 2016 in several European and North African countries. In Tunisia, a sporadic occurrence of wheat stem rust was first reported in 2018 after four decades of absence. Previous Genotyping analysis using SSR markers and race phenotyping based on the Pgt population collected from different geographical locations in 2021 and 2022 in Tunisia revealed the prevalence of three main distinct races (Clade); namely TTRTF (Clade III-B), TKKTF (Clade IV-F), and TKTF (Clade IV-B). Here, we explored the genetic diversity of the Pgt population compared to a global population comprising predominant races from Tunisia, Spain, Italy, and France using whole-genome single nucleotide polymorphisms (SNP) analysis. As a result, the phylogenetic analysis showed that European and North African isolates define a distinct and separated group compared to American and other African isolates. Additionally, the phylogenetic tree revealed that isolates, that were assigned phenotypically the same race, were clustered together, regardless of geographic location. This study provides valuable genomic resources for further studies on the pathogen genome evolution addressing, in particular, the newly re-emerged races.

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## A new automated method for real-time fungal spore quantification.

Julien Alassimone

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Depending on their morphological characteristics, quantifying fungal pathogen spores could be challenging. Automated cell counting devices or flux cytometry techniques are optimized for round-shaped cells. Quantifying *Zymoseptoria tritici*'s spores is mainly done manually using hemacytometer counting chambers. This technique is time-consuming, has low throughput, and does not preserve raw data for later use or validation. The amount of counted spores per sample is limited, and accuracy decreases with operator exhaustion. We developed an automated quantification method based on unbiased automated image analysis. Spore suspensions are loaded in counting chambers and our ImageJ script is accessing the live feed of a microscope camera. Images automatically undergo background removal and detection of non-overlapping spores. Spore detection can be refined using size and roundness exclusion settings. In addition to the quantification results, statistics, settings, image acquisitions, detection overlays and outputs, are saved for quality control and traceability. Those data can be used to re-run analysis or perform downstream analysis. For instance, we developed a phenotyping ImageJ script utilizing the generated outputs to extract phenotyping traits (spore length and branching). In our hands, quantification results benefited from the increase in the counted spore numbers, were generated faster, and gained robustness. One thing is clear: we are not manually counting spores again.

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## TE Hub: a community-oriented space for sharing and connecting tools, data, resources, and methods for transposable element annotation

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Transposable elements (TEs) play a powerful and varied evolutionary and functional role, and are widespread in most eukaryotic genomes. Their role in understanding and annotating genomes has driven the creation of a large collection of databases, software, classification systems, and annotation guidelines. The diversity of available TE-related methods and resources raises compatibility concerns and can be overwhelming to researchers and communicators seeking straightforward guidance or materials. To address these challenges, we have initiated a new resource, TE Hub, that provides a space where members of the TE community can collaborate to document and create resources and methods. The space consists of (i) a website (<http://tehub.org>) organized with an open wiki framework, (ii) a conversation framework via a Slack channel (#te-hub) housed in the larger TransposonsWorldwide workspace, as well as (iii) bi-monthly Hub Update video chats on the platform's development. In addition to serving as a centralized repository and communication platform, TE Hub lays the foundation for improved integration, standardization, and effectiveness of diverse tools and protocols. We invite the TE community, both novices and experts in TE identification and analysis, to join us in expanding our community-oriented resource.

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## A $\beta$ -glucanase from *Z. tritici* releases cell wall-derived immune response elicitors that modulate the outcome of wheat colonization

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The plant cell wall (PCW) is a complex and dynamic structure that plays a crucial role in plant defense against pathogens and is a source of oligosaccharides acting as elicitors of the immune responses. Some pathogenic fungi degrade PCW through the action of a diverse set of secreted cell wall degrading enzymes (CWDEs) that facilitate host colonization. Here, we demonstrate that ZtGH45, a conserved  $\beta$ -glucanase from *Z. tritici*, is induced during the necrotrophic phase and hydrolyzes wheat cell wall polymers, releasing mixed-linked  $\beta$ -1,3/1,4-glucans (MLGs) and cello-oligosaccharides that activate wheat immune responses and hinder pathogen infection. Early misexpression of ZtGH45 leads to an earlier release of MLGs, premature induction of host immunity and impairment of fungal virulence. Altogether, these findings demonstrate that the balance between PCW degradation and the release of resistance inducers by fungal CWDEs might dictate the evolution of regulatory mechanisms governing fungal enzyme expression to promote plant colonization

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# Efficient isolation and transformation protocol of *Zymoseptoria tritici* protoplast from the global reference strain IPO323

Michael Choi

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*Zymoseptoria tritici* is a devastating filamentous fungus and the causal agent of Septoria tritici blotch (STB) in wheat. It has been documented STB could cause up to 50% yield loss in severe conditions while it is also responsible for up to 70% of the total fungicide usage on wheat in Europe. *Z. tritici* has one of the most expansive genome resources for any plant pathogen, with over 1000 publicly available genomes along with over 20 high-quality long-read assemblies. However, our ability to take full advantage of this rich dataset is limited by the lack of a high-throughput method for genetic manipulation of this species. This is especially more complicated in *Z. tritici* as the novel global reference strain IPO323 has anecdotally been reported to be difficult to protoplast. Adding to this difficulty was the only published enzyme (glucanex, Sigma) used to generate *Z. tritici* protoplasts has now been discontinued. Here we developed a protocol for protoplast isolation and transformation of the *Z. tritici* reference IPO323 and other modern *Z. tritici* isolates using the cheap and commercially available wine-making enzyme 'extralyse' (Laffort). We will present an overview of our optimised protoplast isolation method, and transformation efficiency of IPO323 using PEG-mediated transformation.

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## Revealing hidden interactions between blotch disease and other plant pathogen infections

Miguel Ángel Corrales Gutiérrez

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In the field, *Zymoseptoria tritici* is often part a multi-species disease complex generically termed 'leaf blotches'. Furthermore, it can often co-exist with other pathogens sharing the same ecological niches. Recent studies have shown that *Z. tritici* can induce systemic changes in wheat, affecting host immunity to other pathogens. Thus, there is an impact of *Z. tritici* on the host response to secondary infections with pathogens from a different species. While, such questions have been previously studied, the molecular/genetic basis of such phenomena is still poorly understood. Here, we studied a system where *Z. tritici* and different *Fusarium* species causing Fusarium Head Blight (FHB) are co-existing. First, we developed a reductionist approach to simplify the phenotypic readout from such interactions in a simple leaf segment infection assay. Then, we used this set-up to create artificial mycobiome interactions which were then applied to long read, paired-end short read, and small RNA sequencing. In this presentation, we will provide the first insights from this experimental exploration of the interactions between two major wheat diseases, one of them being *Z. tritici*. We will discuss how the ploidy level, and sub-genome composition seem to be genetically shaping the host responses. We also speculate that the observed pattern are highlighting how the host immune system is mitigating important tradeoffs in responses to pathogens with different lifestyles.

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## IDENTIFICATION OF RECEPTORS FOR LEAF BLIGHT PATHOGENS IN WHEAT

Inès Durand

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Bread wheat (*Triticum aestivum*) is a staple cereal crop facing persistent threats from fungal leaf blight pathogens. The fungal pathogen *Zymoseptoria tritici* causes septoria tritici blotch (STB) which is one of the most destructive wheat diseases in Europe. *Ramularia collo-cygni* causing ramularia leaf spot (RLS) is an emerging pathogen of barley. However, reports indicate that *R. collo-cygni* also infects wheat as an alternative host, posing new challenges. Both fungi belong to the Dothideomycetes class. During infection *Z. tritici* and *R. collo-cygni* secrete apoplastic proteins termed effectors to aid infection and manipulate plant immunity. Recent studies in other fungal pathogens suggest that receptor like kinases (RLKs) can be effector targets. Our study aims to identify wheat immune receptors targeted by *Z. tritici* and *R. collo-cygni*. To achieve this, candidate receptors have been identified from the transcriptomic analysis of wheat leaves infected with either *Z. tritici* or *R. collo-cygni* at early time points. Direct interactions between candidate receptors and effectors will be tested with yeast-2-hybrid experiments, followed by co-immunoprecipitation. Given the lack of effective control methods against STB and RLS, there is an urgent need for a better understanding of host-pathogen interactions. By identifying conserved effector targets in wheat, our study aims to provide insights into resistance mechanisms and develop new control methods.

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## Use of Integrated Management Tools to Suppress Tan Spot (*Pyrenophora tritici-repentis*) in Hard Red Spring Wheat in North Dakota

Gabriel Dusek

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Tan spot is an endemic hard red spring wheat (HRSW) disease in North Dakota, USA, and prevalence (number of fields) of the disease in the state has decreased since 2019. Reduction of tan spot has likely been attributed to environmental conditions, frequent use of fungicides, avoiding wheat-on-wheat rotations, and adoption of less susceptible cultivars. To assess the importance of tan spot management, field experiments were established at multiple locations and evaluated fungicide timing and cultivar resistance on reducing tan spot and protecting yield. From 2021 to 2023, nine field experiments were conducted across five locations. Experiments were designed in a randomized complete block, with a split plot arrangement, and four reps. Cultivar served as the main plot and fungicide treatment served as the sub-plot. Three HRSW cultivars included a susceptible, moderately susceptible, and resistant. Four fungicide treatments included a non-treated check, propiconazole applied at the tillering growth stage, prothioconazole+tebuconazole applied at early anthesis, and sequential application of the two fungicides. Very low to low tan spot levels developed on non-treated susceptible checks with none of the experiments documenting economically damaging levels of tan spot. In the low disease experiments, fungicides including the timing of early-anthesis reduced disease pressure on leaves in the lower-third of the canopy, but did not improve yield.

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# Variational autoencoder approach to unravel the sequence semantics of non-coding DNA evolution in fungal genomes

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Non-coding DNA (ncDNA) is known to play pivotal roles in a diversity of biological processes, and gives rise to elements with diverse structures and functions. Despite increasing evidence that genomic variation in ncDNA impacts an organism's fitness, most ncDNA is currently uncharacterized, and low levels of conservation coupled with unknown evolutionary origins makes it particularly difficult to study. DNA shares its sequential property with language, and recently language models have been trained successfully on biological sequences, demonstrating their ability to capture semantic properties in DNA, even though their highly dimensional, sparse internal representations can be difficult to interpret. Variational autoencoders (VAEs) use a probabilistic approach to learn structured, regularized latent spaces, which facilitates model interpretation. Here, we use a VAE coupled with language modelling techniques to investigate properties of coding, functional, and non-functional DNA sequences. We focus on compact fungal genomes, such as *Zymoseptoria triticii*, given the richness and frequency of potential transitions between sequence function in particular along accessory chromosomes and in other rapidly evolving genomic compartments.

# The multifaceted impact of DNA methylation on the major plant fungal pathogen, *Zymoseptoria tritici*

Ivona Glavincheska

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DNA methylation can have multifaceted impacts on genome architecture, stability, and adaptation in eukaryotes. In the case of the wheat fungal pathogen, *Zymoseptoria tritici*, the inactivation of the DNA methyltransferase, Dim2, has resulted in a near-complete loss of cytosine DNA methylation. Interestingly, related species and *Z. tritici* strains from its centre of origin possess an intact copy of Dim2. These strains have an increased mutation rate and a decrease in transposable element (TE) mobility. We aim to understand how loss of Dim2-mediated DNA methylation affects genome architecture, evolvability and TE regulation in *Z. tritici*. We selected eight *Z. tritici* strains to generate Dim2 deletion and complementation transformants. We will use multiomics sequencing to determine how DNA methylation affects the local and global genome architecture. We will then explore if the loss of DNA methylation confers a fitness advantage through TE de-repression in *Z. tritici* strains by evolving the wild-type (WT) and Dim2 mutant strains for 52 weeks under optimal and stressful conditions. In addition, we aim to demonstrate the functional conservation of Dim2 in pre-meiotic and potential mitotic hypermutation of TEs. The combination of experimental evolution, genomics, and epigenomics will highlight the functional importance of intraspecies DNA methylation variation in a wheat pathogen.

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## A wild relative of wheat reveals the significance of lipid transfer proteins in *Zymoseptoria tritici* – plant interactions

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*Zymoseptoria tritici* is a major threat to wheat production. *Aegilops cylindrica*, a wild relative of wheat, is resistant to the wheat-infecting lineage of *Z. tritici* but susceptible to an *Aegilops*-infecting lineage. We constructed a de novo transcriptome assembly of *A. cylindrica* during compatible and incompatible interactions to explore its potential as a novel source of resistance genes against *Z. tritici*. Overall, many genes associated with strong immune responses such as PR-1 were more upregulated during the compatible interaction. However, we observed suppression of several key resistance-associated genes, including homologues of known resistant genes (e.g., RPM1- and RPP13-like) and certain PR genes, including various lipid transfer proteins (LTPs, PR14) and apoplastic subtilisin-like proteases (PR7). Therefore, our study indicates that *Z. tritici* can establish a successful infection in *A. cylindrica*, independent of a strongly activated plant immune response by inhibiting the expression of only a few key immunity-related genes.

## Can Sur7 be used as a protein marker for extracellular vesicles from the fungal wheat pathogen *Zymoseptoria tritici*?

Erin Hill

Australian National University

*Zymoseptoria tritici* is a fungal pathogen responsible for Septoria Tritici Blotch of wheat. As *Z. tritici* is an extracellular pathogen, the apoplast is likely the site of key molecular events underlying disease outcomes. Recent work suggests extracellular vesicles (EVs) may mediate the secretion of defence and virulence molecules during plant–pathogen interactions. We questioned if *Z. tritici* uses EVs to traffic virulence molecules during its infection of wheat. We showed *Z. tritici* produces EVs *in vitro* comparable to other fungal EVs. Proteomics of *Z. tritici* EVs identified ZtSur7, a membrane protein resembling a protein marker for *Candida albicans* EVs. We validated ZtSur7 as *Z. tritici* EV cargo *in vitro* and investigated its use for studying fungal EVs *in planta*. Reverse genetics was used to assess if ZtSur7 contributes to EV secretion and virulence. We concluded ZtSur7 may be a useful tool for *in vitro* studies of *Z. tritici* EVs but not *in planta*.

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## Population genetics of wheat powdery mildew in Europe

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Pathogens responsible for agricultural epidemics evolve rapidly, often acquiring resistance to control methods and rendering them ineffective. Large scale population genomics and molecular epidemiology can help us understand the spread of the disease better and aid in developing more durable management strategies. In this study, we sampled wheat powdery mildew from across Europe and the Mediterranean region over two consecutive years. We used whole-genome sequencing data of 415 isolates collected from over 20 countries to study the population genetics of the pathogen. We find evidence for population structure in the region, with major differences between northern Europe, southern Europe and the Middle East. The population in the north is homogeneous and well-connected while the south appears to be made up of smaller, more differentiated populations. These patterns of genetic diversity are found to be shaped by geography, climate, wind connectivity and potentially also adaptation to hexaploid or tetraploid hosts. Further, genome-wide scans for selection show that different fungicide targets and avirulence loci have been selected in different regions, reflecting the heterogeneity in agricultural practices in Europe and surrounding areas. Finally, we investigate the spatio-temporal dynamics of the disease in light of dispersal rates and previously known instances of resistance-breakdowns in the pathogen.

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## Evolution of gene expression plasticity in *Zymoseptoria tritici*

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The evolution of plasticity has long intrigued scientists who have tried to uncover the intricate mechanisms governing these phenotypic modifications. Theoretical models posit that phenotypic plasticity is likely to evolve in response to fluctuating environments. Nonetheless, empirical evidence from various studies presents a mixed picture, with some studies supporting this prediction while others do not. Here, we use experimental evolution tools to study the evolution of gene expression plasticity in strains of *Zymoseptoria tritici* exposed to stable and fluctuating environmental conditions. Here, we aim to study the evolution of transcriptome after exposure to variable environments for over 200 generations. We generate a full-factorial combination of experimental treatments, combined with stable and fluctuating regimes. Our plan is to measure the change in the gene expression after exposure to the experimental treatments and analyze if certain regime (stable or fluctuating) results in evolution of gene expression plasticity in the strains. Furthermore, we would assess the changes in gene regulatory networks across replicates and analyze if the evolution of gene expression patterns is repeatable. Exploring interactions between abiotic factors like temperature and media composition helps us understand how strains respond to environmental stimuli and whether this response is mediated through shared or distinct pathways.

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## Deciphering the dynamics of 3D genome organization in *Zymoseptoria tritici*

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In Eukaryotes, the 3D genome organization is involved in major biological processes, such as gene expression and DNA replication. However, the genomic organization remains poorly understood in fungi. *Zymoseptoria tritici*, the fungal pathogen leading to the Septoria tritici leaf blotch (STB) in wheat, presents a massive intra-species diversity in terms of genomic content, accessory chromosomes, and transposable elements. Interestingly, core and accessory chromosomes show similar genomic content, but are not enriched with the same histone post-translational modifications. One way to better understand the general 3D features of the species is by performing a High-throughput sequencing coupled with Chromosome Conformation Capture (Hi-C) method. Moreover, it allows to link this conformation with different genomic scales, such as histone modifications, trans-expression Quantitative Trait Loci (eQTL), structural variants and gene expression. Doing Hi-C with different strains helps to investigate the role of the 3D structure linked to the high genomic variability of the species.

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## Fungicides alternation and mixture lead to *in vitro* selection of generalist resistance mechanisms (MDR) in *Zymoseptoria tritici*

Anaïs Lalève

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Recent experimental evolution studies under various fungicide application conditions on *Zymoseptoria tritici* have led to the selection of resistant strains. Some of them display MDR phenotype, which are not linked to known MFS1 expression regulation. This suggests that previously undescribed MDR mechanisms may have been selected through these experimental evolutions. To elucidate whether increased efflux is involved in these phenotypes, efflux tests were carried out using a fluorescent molecule or the interaction between terbinafine and transport modulators on fungal growth. These efflux assays suggest that for some strains, their MDR phenotype may be attributed to increased fungicide efflux whereas for others alternative MDR mechanisms may be involved, not necessarily through increased efflux. Whole-genome sequencing data provided some candidates currently under functional validation may provide further insights into the molecular mechanisms underlying MDR.

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## Establishment of pangenome graphs for the analysis and monitoring of plant pathogen populations

Nicolas Lapalu

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The assembly of whole genomes by long-read sequencing has enabled the collection of resolved telomere-to-telomere (T2T) haplotypes, encouraging genome-wide approaches to study structural variations. Unlike comparative genomics approaches based on gene content or other types of annotation (transposable elements), the analysis of structural variants in genome-wide analysis is now commonly based on graph-oriented approaches. The pangenome graphs (PGG) are capable of storing the full genetic diversity of the genomes under study, achieving superior performance in read alignment, variant identification and genotyping for population studies. We propose to establish a pangenome graph for *Zymoseptoria tritici* with the available genomic sequences as well as 2 new assemblies of strains isolated on durum wheat (*T. turgidum* var. durum). Our goal is to evaluate the use of PGG instead of linear-reference genome for GWAS and GEA analyses and implement process to modify and improve the graph with upcoming new data. Establishing a shared reference PGG based on FAIR principles could be valuable to the community, such as the IPO323 reference genome. In addition, same analyses will be carried out with *Pyricularia oryzae* to validate the method and propose a common framework for other fungal species. We will present our first results on the establishment of graphs and the detections of variants compared to previous results obtained from single reference genome.

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## Atypical isolates of *Pyrenophora tritici-repentis* from Argentina

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*Pyrenophora tritici-repentis* (Ptr) causes tan spot, an important foliar disease of wheat worldwide. Different races of the fungus produce various necrotrophic effectors (NEs) that induce chlorosis or necrosis on specific host genotypes. In this study, a collection of Ptr isolates from eight different localities in Argentina underwent phenotypic race classification using a host differential set. Additionally, isolates were assessed for the presence of NE genes, including ToxA, ToxB, toxB, and ToxC1, by PCR analysis. Based on their virulence phenotypes, races 1, 2, 7, and 8 were identified, with the latter being predominant. Notably, race 7 was detected for the first time in Argentina. However, the phenotypic race classifications of many isolates did not match their respective complement of NE genes, as determined by PCR. These atypical isolates induced chlorosis on either or both 6B662 and 6B365 but lacked the expected genes ToxB, toxB and ToxC1, respectively. The absence of these genes in isolates that nonetheless caused chlorosis suggests the presence of additional NEs, and underscores the need for further investigation.

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## Understanding the mode of action of 4-Phenylbutyric Acid: a lead for sustainable agriculture

Amandine Le Ruyet

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4-phenylbutyric acid (4-PBA) is bacterial metabolite with biocidal activity towards 12 Ascomycota and 2 Oomycota species. It has also a potential plant defence stimulation (PDS) activity. To optimize the development and predict the durability of this promising biocontrol fungicide, we endeavour to elucidate its double molecular mode of action in the *Zymoseptoria tritici* / *Triticum aestivum* pathosystem. We thus selected fungal mutants resistant to 4-PBA by means of *in vitro* directed evolution and established their cross-resistance profiles, including EC50. 4-PBA-specific, highly resistant isolates were sequenced and the comparison of their whole-genomes with those of the parental strains should allow us to identify potential targets of 4-PBA. In addition, *in planta* tests were carried out to validate 4-PBA efficiency under controlled conditions.

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## Unravelling molecular mechanisms involved in overcoming Stb16q wheat resistance to *Zymoseptoria tritici*

Marc-Henri Lebrun

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At least 23 Stb genes control the resistance of wheat to *Zymoseptoria tritici*. All Stb genes have been overcome by virulent isolates. Stb16q incorporated in cultivar Cellule released in France in 2013 was then efficient against all *Z. tritici* isolates. However, by 2016, the first virulent isolates were detected in Northern France and since, in all France and in Europe. We have used cross between a virulent isolate (CFZ8) and an avirulent isolate (IPO323) to identify genes responsible for virulence on Cellule. Phenotypic analysis of this progeny revealed the segregation of a single gene controlling the virulence on these on Cellule and the Chinese spring line carrying only Stb16q (Cs+16). QTL mapping with GBS markers identified a 60 Kb locus on chromosome 11 carrying the gene controlling virulence on Cellule and Cs+16. At this locus, six genes encoding effectors were identified. Functional assessment of these genes included complementation of the virulent parent with the avirulent haplotype and targeted deletion in the avirulent parent. One of these candidate genes behaved as an avirulence gene, called Avr-Stb16q. These results will help studying mechanisms involved in the gain of virulence on Stb16q, and recognition of Avr-Stb16q isolates by Stb16q wheat cultivars.

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## Unraveling the role of a plant cell wall degrading enzyme of *Zymoseptoria tritici* during wheat infection

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*Zymoseptoria tritici* is a latent necrotrophic pathogen that infects wheat and grows in the apoplastic space in close contact with plant cell walls (PCWs). These complex and dynamic structures play a crucial role in plant defence against *Z. tritici* and are speculated to constitute a barrier that prevents pathogen penetration to the host. Additionally, PCWs are a source of oligosaccharides that may act as elicitors of the host immune response. To facilitate its colonization, *Z. tritici* degrade PCW compounds through the action of a diverse set of secreted cell wall degrading enzymes (CWDEs). The genome of *Z. tritici* harbours 203 putative glycoside hydrolases (GHs) with potential CWDE activity. Here, we demonstrate that ZtGH54, encoding a predicted  $\alpha$ -L-arabinofuranosidase, has a peak of expression at the beginning of the necrotrophic phase and that ZtGH54 lack-of-function mutants are more virulent to wheat. The potential oligosaccharides released by ZtGH54 from the wheat cell wall, xylotetraose and 33- $\alpha$ -L-Arabinofuranosyl-xylotetraose, activate wheat immune responses and enhance resistance against *Z. tritici* infection. These results suggest that ZtGH54 activity leads to the production of host immune response elicitors. We hypothesize that despite the negative effect of ZtGH54 in *Z. tritici* infection, it should play a critical role in nutrient acquisition, since it is highly conserved in *Z. tritici* populations.

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## Avr-R interactions contribute to non-host resistance of wheat against non-adapted *Zymoseptoria* species

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Resistance of an entire plant species against all strains of a pathogen species is known as non-host resistance (NHR) and considered more durable than race-specific resistance. These two types of resistance are generally thought to be governed by distinct mechanisms. However, recent work in our lab and others suggested that Avr-R interactions, a hallmark mechanism of race-specific resistance, might also contribute to NHR. We use the wheat pathogen *Zymoseptoria tritici* and its closest relatives, which are not adapted to wheat, to study the contribution of Avr-R interactions to NHR. When expressed in *Z. tritici*, homologs of Avr3D1 and AvrStb9 from both *Z. pseudotritici* and *Z. ardabiliae* induce resistance in wheat in a cultivar-specific manner, indicating a gene-for-gene interaction. Furthermore, deletion of an Avr3D1 homolog in *Z. ardabiliae* led to the formation of asexual fruiting bodies on wheat, which was never observed in the wild type. These results indicate that NHR can be governed by Avr-R interactions and that loss of Avr genes might contribute to the emergence of new diseases. As a complementary approach to discover Avrs involved in NHR and other non-host determining factors, we are currently performing a forward genetic screen and experimental evolution to identify mutants of non-adapted species that can overcome wheat resistance.

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## Unraveling the genomic basis of *Zymoseptoria tritici* Pathogenicity on wheat cultivar Extase

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*Septoria tritici* leaf blotch (STB) disease, caused by the fungal pathogen *Zymoseptoria tritici*, poses a significant threat to wheat production globally. In this study, we aimed to elucidate the genetic determinants underlying the pathogenicity of *Z. tritici* on the wheat cultivar Extase. In the current project, we collected 214 *Z. tritici* isolates from commercial wheat varieties in Ireland and the United Kingdom between 2016-2022. Through infection assays and subsequent genomic sequencing, we identified 138 isolates with distinct pathogenic profiles on cv. Extase. Leveraging bioinformatic tools, we conducted a comprehensive analysis of the sequenced genomes, unveiling significant single nucleotide polymorphisms (SNPs) associated with pathogenicity. Notably, our genome-wide association study (GWAS) pinpointed lead SNPs predominantly located on chromosome 2, suggesting key genomic regions involved in the interaction between *Z. tritici* and cv. Extase. Furthermore, through expression profiling and phylogenetic analysis, we identified candidate genes potentially influencing the pathogenicity of *Z. tritici* on Extase.

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## First Molecular Detection of Propiconazole Resistance in Iranian *Zymoseptoria tritici* Isolates: Identification of CYP51 Gene Mutations

Amir Mirzadi Gohari

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Fungicide resistance poses a significant challenge in controlling *Septoria tritici* blotch (STB) worldwide, a highly destructive disease in wheat-growing regions that can lead to substantial crop losses of up to 50%. The causative agent, *Zymoseptoria tritici*, exhibits a remarkable evolutionary capacity. The declining efficacy of certain azole fungicides in combating *Z. tritici* is attributed to the selection and dissemination of specific mutations in the CYP51 gene within the pathogen population. Unfortunately, the widespread usage of C-14 demethylation inhibitors (DMIs), the most prevalent fungicides, has led to the emergence of resistant strains to azoles. In this study, we investigated the resistance of Iranian *Z. tritici* isolates against the commonly employed fungicide, Propiconazole, and identified the predominant molecular mechanism associated with resistance in the targeted isolates. Following the mode of action of azole fungicides, we amplified and sequenced the gene and promoter of CYP51, which encodes the enzyme 14 $\alpha$ -demethylase, a member of the Cytochrome P450 superfamily. Studying recent isolates validates the presence of certain combined mutations linked to azole resistance. Notably, three novel mutations, G450R, L4V, and E454K, were observed in fungicide-resistant *Z. tritici* isolates. Ongoing investigations include assessing the expression level of CYP51 in the resistant isolates through quantitative RT-PCR.

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## Unveiling adaptive mechanisms of *Zymoseptoria tritici* to climatic conditions

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Fungal plant pathogens pose significant threats to food security and are dominant components of global agroecosystems. Despite wide environmental distributions, many fungal populations are locally adapted to cultivated hosts, fungicide uses, and climatic conditions. Understanding the mechanisms through which pathogens overcome control measures and acclimate to new environments is essential for predicting the future impact of crop diseases. Our study focused on *Zymoseptoria tritici*, which causes the Septoria tritici blotch (STB) disease, representing one of the main constraints on wheat production worldwide. The control of STB is increasingly challenging due to *Z. tritici*'s rapid evolution and adaptation to environmental conditions. We addressed this challenge by leveraging Illumina sequencing data obtained from 240 *Z. tritici* isolates sampled from eight Euro-Mediterranean countries, representing the diverse environmental heterogeneity of wheat-growing regions. Through Genotype-Environment Association (GEA) and Redundancy Analyses (RDA), we identified candidate genes linked to *Z. tritici*'s adaptation to diverse climatic conditions. Furthermore, our study revealed discernible signatures of selection at both the genome-wide and candidate gene levels, further enriching our understanding of the pathogen's evolutionary trajectory. This extensive population genomic analyses underscores key genetic determinants involved in the adaptive potential of this important wheat pathogen.

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## DMI fungicide driven selection in *Zymoseptoria tritici* is independent of geographical and genetic background of the pathogen

Eula Gems Oreiro

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*Zymoseptoria tritici* is an important plant pathogen responsible for septoria tritici blotch (STB) on wheat crops worldwide. Demethylation inhibitor (DMI) fungicides are commonly used to manage STB by targeting the synthesis of sterol 14- $\alpha$ -demethylase which is crucial for pathogen cell permeability and encoded by the CYP51 gene. However, prolonged DMI usage has led to *Z. tritici* populations developing reduced sensitivity to this fungicide group. In this study, 311 isolates were collected pre-treatment in nine wheat growing regions in Europe in 2019. High-throughput amplicon based sequencing of nine housekeeping genes and the CYP51 gene was used to analyze these isolates. Minimum spanning network analysis of the housekeeping gene data showed no population structure among *Z. tritici* samples. We identified several mutations in the CYP51 that were independent of geographical origin. These mutations were combined in different haplotypes most of which had been previously reported as being linked to fungicide resistance. A clear clustering of CYP51 haplotypes was found, indicating selection pressure from DMI use. Overall, these findings show the potential of high-throughput sequencing in monitoring fungicide resistance to support the development of effective and sustainable anti-resistance strategies.

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## What is the impact of varietal mixtures on the frequencies of virulence in *Zymoseptoria tritici* populations ?

Chloé Papin

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Septoria Tritici Blotch (STB) caused by *Zymoseptoria tritici* is a major foliar disease of wheat. Disease control is generally undertaken by application of fungicides and use of varieties carrying resistance genes (Stb genes). But these control methods can be quickly overstepped as they impose high selection pressure on the pathogen, especially when deployed widely and uniformly. Mixing different varieties in field offers an alternative deployment of Stb genes. The objective of this work (PhD Arvalis-INRAE) is to characterize the impact of varietal mixtures on STB. We aim at elucidate the epidemiological processes underlying the interaction between *Z. tritici* and wheat varietal mixtures. To this end, we selected three bread wheat varieties, each carrying different Stb genes. These varieties are cultivated in pure, binary, and ternary mixtures within microplots inside two experimental designs. One design is composed of 48 microplots, half of them inoculated with a specific strains of *Z. tritici*, and is repeated on two sites. We will monitor the change in frequencies of virulence throughout the epidemic. This will be achieved by sequencing avirulence genes in fungal populations and estimating the frequency of virulence against Stb genes by bulk phenotyping. These experimental designs should enable us to estimate the impact of varietal mixtures on the durability and efficacy of Stb resistance, by monitoring the variations of virulence frequencies in the population of *Z. tritici*.

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## Mechanisms for adaptation and increased virulence of Australian *Parastagonospora nodorum* pathogen of wheat

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*Septoria nodorum* blotch is an economically important fungal disease of wheat caused by *Parastagonospora nodorum*. The fungus expresses several necrotrophic effectors that cause necrosis and/or chlorosis on hosts carrying matching dominant susceptibility genes. In this study, we assembled a panel of 360 *P. nodorum* strains consisting of 260 historic and 100 temporal isolates and projected their population structure using 18,209 biallelic SNP data-set covering their entire genomes. This *P. nodorum* population consists of 8 subpopulations with one core found throughout the collection location and time and 7 non-core groups that are transient and emergent found in restricted locations and time. This finding confirmed what was previously reported and revealed the same pattern of low-amplitude boom-and-bust cycles in the temporal population. The newly emerged groups are more pathogenic on modern wheat elite lines and drive adaptation of the pathogen population. For the first time, this study identified mechanisms of the constant emergence for the adaptation where isolates from the core group evolved to form its own lineages using sexual and parasexual reproduction. This research also elucidates what drives variation in their virulence. In my talk, I will present analysis on the population's diversity/structure, their effector and reproduction patterns, new findings on mechanisms for adaptation and explain how they become more aggressive on Australian wheat elite cultivars.

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## Maintaining fungicide effectiveness: Monitoring local *Zymoseptoria tritici* populations for sensitivity shifts and mutations in Estonian fields

Regina Pütsepp

Centre of Estonian Rural Research and Knowledge

A decrease in effectiveness to control *Septoria tritici* blotch has been observed in Western Europe, while in North-East Europe including Estonia, sensitivity to fungicides tends to be relatively high. It is crucial to monitor the local *Z. tritici* population for mutations in fungicide adaptation and shifting sensitivity to detect changes before field performance declines. Our study assessed the sensitivities (EC50) of over 800 isolates in Estonia in six years (2018-2023) and compared them with prevalent mutations in fungicide target sites (SDH, CYP51, CytB). We tested several active substances of DMIs, SDHs, Qols, and a new Qil (fenpicoxamide). Sensitivities to most of the tested active substances were high, except for Qols associated with a high G143A mutation frequency (around 80%) in CytB. The CYP51 gene is frequently mutated, with mutations D134G, V136A and A379G occurring in isolates with a frequency of 20-40% depending on the year. Almost every isolate has a mutation I381V, and the frequency of mutation S524T has increased from 10% to 32% of the total population. Although mutations in SDH subunits are rare, isolates with mutations B-N225I, B-T268I, C-W80S, C-N86K, or C-H152R are usually present, accounting for up to 5% of the population. In 2023, there was a rapid increase of isolates with C-N86S mutations, which accounted for up to 25% of the population. Overall, these findings suggest that CYP51 and SDH genes are important targets for mutations risk surveillance.

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## Identification of a receptor kinase-leucine rich repeat as a strong candidate for the tan spot susceptibility gene Tsc1 in wheat

Katherine Running

North Dakota State University

The necrotrophic fungal pathogen *Pyrenophora tritici-repentis* (Ptr) causes the foliar disease tan spot in bread and durum wheat. Wheat lines with the tan spot susceptibility gene Tsc1 are susceptible to Ptr ToxC-producing isolates, which display chlorosis. Previously, Tsc1 was mapped to a 1.4 cM genetic interval spanning 184 kb on chromosome arm 1AS. Inoculations of sequenced wheat lines with a Ptr ToxC-producing and a Ptr ToxC-disrupted isolate demonstrated that the variety CDC Landmark developed Ptr ToxC-dependent chlorosis, indicating the presence of a functional Tsc1 allele. The chlorosis phenotype mapped to the Tsc1 locus in a doubled haploid population derived from CDC Stanley x CDC Landmark, further confirming the presence of Tsc1 in CDC Landmark. Therefore, the CDC Landmark genome was used in addition to the Chinese Spring reference genome to evaluate Tsc1 candidate genes. Comparative analysis of candidate genes in the two genomes reduced the candidates to two genes. Sequence analysis of five chemically-induced mutants revealed that a receptor kinase with protein kinase and leucine-rich repeat domains was necessary for chlorosis induction, making it a strong candidate for Tsc1. Preliminary haplotype analysis indicates that in many chlorosis non-producing lines Tsc1 is absent. The map-based cloning of Tsc1 provides a foundation for functional characterization of Tsc1 and the development of diagnostic Tsc1 markers to aid in the production of tan spot-resistant wheat.

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## Characterization of wheat genes governing sensitivity to the *Parastagonospora nodorum* necrotrophic effector SnTox5

Katherine Running

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The necrotrophic fungal pathogen *Parastagonospora nodorum* produces necrotrophic effectors (NEs) that target specific genes in wheat to cause the disease septoria nodorum blotch (SNB). The interaction between the NE SnTox5 and the wheat gene *Snn5* plays a significant role in the development of SNB. *Snn5* was previously mapped to chromosome arm 4BL. Here, we combined positional cloning, TILLING, and gene editing approaches to clone *Snn5*. *Snn5* encodes a protein with protein kinase and major sperm protein (PK-MSP) domains. Analysis of *Snn5*-disrupted mutants indicated that both major domains are essential for function. Through related genetic experiments, we identified a second SnTox5-sensitivity locus on chromosome arm 2BS. This gene was delineated to an 8.54 cM interval corresponding to a 9.6 Mb region in Svevo Rel 1.0 containing six PK-MSP-like genes. Given the presence of multiple SnTox5 sensitivity genes, we propose that the original sensitivity gene be termed *Snn5-B1* and the second sensitivity gene mapped to chromosome 2B be termed *Snn5-B2*. Genome-wide association studies on large panels of durum and common wheat indicated that *Snn5-B1* was predominant among common wheat accessions whereas *Snn5-B2* was relatively more frequent among durum lines. This research extends our knowledge of the *Snn5*-SnTox5 interaction and the wheat-*P. nodorum* system in general, and provides tools to aid in the development of SNB-resistant wheat via marker-assisted selection and gene editing.

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## Discovery of novel phytotoxins from the wheat tan spot fungal pathogen *Pyrenophora tritici-repentis*

Pao Theen See

Curtin University

*Pyrenophora tritici-repentis* (Ptr) is the causal agent of the tan spot disease that affects global wheat production. Ptr, known as a necrotroph and /or a narrow host range polymertroph secretes multiple effectors to facilitate host colonisation. ToxA and ToxB are both well-characterised proteinaceous effectors while the structure of ToxC, a low-molecular weight effector molecule remains to be elucidated. Despite the advances in our understanding of protein effector biology, we still have much to explore as the mechanisms of infection are extensive and involve not only secreted proteins but also small metabolites. Unlike effector proteins, studies on metabolites are more challenging because of the wide range of possible chemical structures. Moreover, these compounds are commonly present in complex biological mixtures and in low abundance, which are unfavourable conditions in purification processes. Here, we present the discovery of two bioactive novel secondary (or specialised) metabolites from Ptr. Both compounds induce chlorosis in a cultivar-specific manner but with different intensity levels. Response of chlorosis was light-dependent and one compound exhibited phytotoxicity on non-host plants. Neither compound has properties consistent with ToxC. We postulate that these compounds may have an ancillary role in disease development.

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## QTL mapping for temperature tolerance the wheat pathogen *Zymoseptoria tritici*

Jessica Stapley

ETH Zurich

Global warming is expected to have adverse impacts on global agriculture, as it influences plant disease occurrence and severity. Understanding the genetic basis of adaptation to temperature in fungal plant pathogens is crucial to predict how pathogen populations will respond to warming climates and how they may impact agricultural systems in the future. Temperature can influence fungal fitness in multiple ways, by directly influencing their growth, virulence and reproduction, and also indirectly by influencing plant immune responses. In this study we combine QTL crosses and a large phenotypic and genomic dataset to identify large effect loci associated with tolerance to temperature stress in *Z. tritici*. QTL mapping was performed in crosses established between strains collected in Switzerland. Size and melanin were measured *in vitro* at 8 and 12 days post inoculation, at 10, 18 and 27 degrees Celsius in 259 and 265 offspring from two QTL crosses. We identified QTL peaks specific to temperature stress that contained several promising candidate genes, including a heat shock protein (Hsp90).

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## Identification of QTL associated with *Septoria tritici* blotch resistance, in the winter wheat population, in Ontario, Canada

Ljiljana Tamburic-Ilincic

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*Septoria tritici* blotch (STB) is an important wheat disease in North America. Yield losses worldwide range from 5-10%, when resistant cultivars are planted and fungicides are applied, to 50% in fields planted with susceptible wheat cultivars. The objective of this study was to map loci associated with STB and plant height in a Maxine/FTHP Redeemer winter wheat population. Evaluation of STB resistance was performed using spray inoculation of a mixture of *Zymoseptoria tritici* isolates and under natural infections, in replicated trials across three environments in Ontario, Canada. The population showed a continuous distribution pattern. Quantitative trait loci (QTL) analysis was performed by evaluating 105 doubled-haploid lines. QTL for STB were identified on chromosome 2D, 4B (derived from FTHP Redeemer) and 7A (derived from Maxine). QTL for plant height was also identified on chromosome 4B. Significant negative correlation was recorded between STB and plant height and STB and heading date. The pleiotropic effects of the Rht genes on plant height and disease resistance is an issue in breeding programs. Introduction of these alleles, in the future lines, will improve resistance of wheat cultivars to STB.

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## Transcriptomic analysis revealed compensatory pathogenicity mechanisms expressed in *Parastagonospora nodorum* lacking major necrotrophic effectors

Kar-Chun Tan

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The fungus *Parastagonospora nodorum* causes septoria nodorum blotch (SNB) of wheat by secreting a suite of proteinaceous necrotrophic effectors (NEs) to induce tissue necrosis upon infection. Tox effectors only induce necrosis/chlorosis on wheat cultivars that possess matching dominant susceptibility genes (Snn). It has been demonstrated that multiple NE-Snn interactions dictate the outcome of SNB through additive but also epistatic interactions. In this study, we have generated a *P. nodorum* mutant (toxa13) that lacked major NE genes; ToxA, Tox1 and Tox3. Surprisingly, the virulence *P. nodorum* toxa13 is comparable to the wildtype on modern bread wheats despite ablating three NE-Snn interactions. This suggests that other functionally redundant pathogenicity mechanisms compensate for the loss of the three major effectors. A comparative RNASeq study then revealed that effector gene Tox267 and two phytotoxic secondary metabolism (SM) gene clusters were highly up-regulated in toxa13 in-plant. Furthermore, several candidate NE genes, uncharacterised SM gene clusters and signal transduction genes were also found to be up-regulated and may contribute to maintaining the virulence of toxa13. Characterisation of these genes for their role in the sustained virulence of *P. nodorum* toxa13 will be discussed.

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## Within field population diversity of wheat pathogen *Zymoseptoria tritici*

Andrea Tobian Herreno

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*Zymoseptoria tritici* is a cosmopolitan hemibiotrophic wheat pathogen with a high mutation rate and a mixed reproduction system, leading to challenges in traditional farming management. For successful integration of pest management, especially for early diagnostics of new aggressive or fungicide-resistant lineages, it is critical to understand population diversity in the field and develop efficient and adequate population sampling strategies. We deeply characterise population structure and diversity features, such as minor allele frequency distribution and clonality by assessing data from different *Z. tritici* field populations. We specifically look at the effects of NGS data filtering in terms of confidence intervals for calling an alternative allele, minimum genotyping rate, maximum missing data allowed for the call, and enforcing biallelic and non-biallelic output, among others. We apply these analyses to different datasets to differentiate within field diversity in similarly-sized fields from three different locations: the United Kingdom, the United States, and Switzerland. We show general differences between the European and US fields and the effect of different sampling strategies. These insights will help us develop better sampling strategies to maximize the capture of diversity at the field level.

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## Understanding the structural basis of apoplastic effector recognition by wall-associated kinases

Simon Williams

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Recognition of apoplastic effectors by extracellular plant receptors is crucial for disease resistance and susceptibility in cereal crops. We are interested in understanding the structural basis of these receptor-effector interactions, with a particular focus on Wall-associated kinase (WAK) mediated resistance and susceptibility. Here, I will present data detailing the protein production systems that we have established to study WAK – effector interactions. I will also present preliminary biophysical and structural data based on our studies.

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