# International Symposium on Cereal Leaf Blights (ISCLB) 2019: Book of Abstracts

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<tr>
<td><strong>Authors(s)</strong></td>
<td>Feechan, Angela; Benbow, Harriet; Tiley, Anna; Gibriel, Hesham; Casey, Edward; Doohan, Fiona M.</td>
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<tr>
<td><strong>Publication date</strong></td>
<td>2019-04-24</td>
</tr>
<tr>
<td><strong>Conference details</strong></td>
<td>Dublin, Ireland</td>
</tr>
<tr>
<td><strong>Publisher</strong></td>
<td>University College Dublin</td>
</tr>
<tr>
<td><strong>Link to online version</strong></td>
<td><a href="https://www.isclb2019.com/portal/public/abstracts/">https://www.isclb2019.com/portal/public/abstracts/</a></td>
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<tr>
<td><strong>Item record/more information</strong></td>
<td><a href="http://hdl.handle.net/10197/11177">http://hdl.handle.net/10197/11177</a></td>
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International Symposium on Cereal Leaf Blights (ISCLB) 2019

University College Dublin
22 to 24 May 2019

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Host Genetics and Resistance Breeding

Host-Pathogen Interactions

Pathogen Functional Genetics and Genomics

Oral Presentations

Evolution and Population Biology

DNA methylation impacts genome evolution of Zymoseptoria tritici

Dramatic recent changes in the population genetic diversity of the Zymoseptoria tritici effector gene AvrStb6

Hectic Life on Wheat Leaves: Dynamics of Phenotypic Selection within Zymoseptoria tritici Populations Facing Microclimatic Heterogeneities

Cultural Management, Fungicide Resistance and Epidemiology

Independent emergence and spread of azole fungicide resistance in the wheat pathogen Zymoseptoria tritici in Australia

The impact of barley variety rotation, mixtures, and intercropping on leaf disease and silage production

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Methylation of DNA is an important component of epigenetic regulation and found throughout all forms of life ranging from prokaryotes to mammals. However, the extent and function of DNA methylation differ between taxa. Previous studies in the plant pathogenic fungus *Zymoseptoria tritici* report absence of DNA methylation in the reference strain IPO323 due to amplification and inactivation of the DNA methyltransferase gene *Ztdim2* by repeat-induced point mutations (RIP). In this study, we demonstrate that *Ztdim2*, however, is not inactivated in other strains of *Z. tritici* that have maintained a functional *Ztdim2* gene. We used bisulfite sequencing to identify genome wide cytosine methylation levels in strains with and without a functional *Ztdim2*. The presence of a functional *Ztdim2* correlates with high levels of cytosine methylation on transposable elements indicating a role in the genome defenses. We present evidence for the presence of small amounts of DNA methylation, even in strains containing an inactive *Ztdim2* gene suggesting that DNA methylation was maintained over time. This scenario is supported by the presence of a putative maintenance DNA methyltransferase, *Ztdnmt5* in the *Z. tritici* genome. Integration of a functional *Ztdim2* variant in strains with inactivated *Ztdim2* restores DNA methylation levels indicating de novo methylation activity of *Ztdim2*. We compared the genomes of strains that maintained high levels of DNA cytosine methylation with genomes of strains without *Ztdim2* activity. We found that the presence of DNA methylation alters nucleotide composition by promoting C→T transversions and thereby likely contributes to transposon inactivation and influences genome evolution dynamics. Taken together, our results indicate that the presence of widespread DNA methylation is a variable trait in *Z. tritici* populations that impacts genome evolution as a mechanism of genome defense.
Dramatic recent changes in the population genetic diversity of the *Zymoseptoria tritici* effector gene *AvrStb6*

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*Zymoseptoria tritici*, the causal agent of Septoria tritici blotch (STB), is among the most commercially important global pathogens of wheat. Recently, both genetic factors of the previously characterised gene-for-gene interaction between *Z. tritici* and wheat have been identified: the wheat receptor-like kinase Stb6 and the *Z. tritici* secreted protein AvrStb6. Subsequently, historic collections of *Z. tritici* collected around the world have been used to analyse *AvrStb6* genetic diversity. However, which of the identified *AvrStb6* haplotypes confer virulence on wheat possessing Stb6 and the frequency of virulence in the global populations remain unknown. Here, we re-sequenced *AvrStb6* from recent field populations of *Z. tritici* collected between 2014 and 2017 from Western Europe, Turkey, USA, South America, and Australia.

As well as *AvrStb6* being present in all isolates tested, we found a small number of haplotypes, all encoding the same protein isoform conditioning virulence on Stb6-containing wheat, predominate in different parts of the world. The avirulence isoform of AvrStb6, first used to characterise the gene-for-gene interaction in this pathosystem, was not detected. This contrasts with findings from a study of a global *Z. tritici* population sampled between 1990 and 2001 (Brunner & McDonald, 2018) that identified a high *AvrStb6* haplotype diversity and no clear dominance of a single haplotype, and a study of a *Z. tritici* population sampled between 2009-2010 predominantly from France where the avirulence isoform was detected in ~20% of isolates (Zhong et al. 2017). It would appear that a significant shift in the global *Z. tritici* populations, towards a single Stb6 resistance-breaking isoform of Avrstb6, has taken place in recent years. We hypothesise that selection pressure imposed by Stb6 haplotypes present in many contemporary commercial wheat cultivars may be responsible for this shift. This work will help to pinpoint specific amino-acid changes in AvrStb6 that allow evasion of Stb6 detection.

References:


This abstract was presented orally at 11:00 on Wednesday 22 May 2019
Hectic Life on Wheat Leaves: Dynamics of Phenotypic Selection within *Zymoseptoria tritici* Populations Facing Microclimatic Heterogeneities

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Wheat foliar pathogens inhabit crop canopies that are subjected to substantial spatio-temporal variations in temperature. Given the pronounced diversity in thermal individual responses within a local *Zymoseptoria tritici* population, these environmental heterogeneities may lead to phenotypic selection in the field. Signatures of response to thermal selection over the course of annual Septoria tritici blotch (STB) epidemics have been previously identified. However, the consequences on population dynamics of the interplay between interindividual phenotypic variation and thermal canopy heterogeneity have not yet been investigated. We address this issue using a three-step approach. First, we quantified the extent of environmental (measurement of temperature distribution within wheat canopies) and phenotypic (standardised thermal phenotyping of *Z. tritici*) variations encountered by or in natural pathogen populations. Second, we dissected the processes underlying spatio-temporal changes in the phenotypic composition of populations facing different types and extent of microclimatic heterogeneities by performing *in planta* polycyclic selection experiments (growth chamber and field experiments). Third, we explored *in silico* the way in which phenotypic diversity affect population adaptive dynamics (quantitative assessments of population vulnerability and resilience to thermal changes). Our results show how environmental signals, interindividual phenotypic variations and ecological processes have affected *Z. tritici* population dynamics in our experiments. In particular, three major findings emerged from this investigation: (i) the occurrence and the epidemiological consequences of short-term selection driven by seasonal temperature variations over an annual epidemic; (ii) the critical importance of spatio-temporal thermal heterogeneity in wheat canopies in the maintenance of phenotypic diversity within *Z. tritici* populations through the presence of thermal refugia; (iii) the quantitative impacts of oversimplifications currently adopted in disease prediction models. By shedding new light on population adaptive potential to environmental variations, these insights would help to improve predictions of the eco-evolutionary responses of populations to changing climate.

This abstract was presented orally at 11:20 on Wednesday 22 May 2019
Independent emergence and spread of azole fungicide resistance in the wheat pathogen \textit{Zymoseptoria tritici} in Australia

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\textit{Zymoseptoria tritici} is a globally distributed fungal pathogen, which causes Septoria tritici blotch on wheat. In the absence of resistant cultivars, crop protection is achieved through the application of fungicides. However, fungicide resistance is commonly observed in \textit{Z. tritici} populations and continuous monitoring is required to detect breakdowns in fungicide efficacy. We recently reported the discovery of azole resistant isolates in Australia. However it remained unknown whether resistance was brought into the continent through gene flow or whether resistance emerged independently. To address this question, we screened 43 isolates across 5 Australian locations for azole sensitivity and performed whole genome sequencing on 58 isolates from seven locations to determine the genetic basis of resistance. Population genomic analyses showed extremely strong differentiation between the post-azole Australian population and both pre-azole Australian populations and populations on different continents. The apparent absence of recent gene flow between Australia and other continents suggests that azole fungicide resistance has evolved \textit{de novo} and subsequently spread within Tasmania. Despite being distinct at the whole genome level, we observed identical combinations of non-synonymous substitutions at the \textit{CYP51} locus as observed elsewhere in the world. We observe nine previously reported non-synonymous mutations including isolates that carried a combination of the L50S, S188N, A379G, I381V, Y459DEL, G460DEL, N513K substitutions. \textit{EC}_{50} assays for a subset of isolates exposed to tebuconazole and epoxiconazole fungicides showed high levels of azole resistance. Since this discovery we have monitored the spread of these mutations to continental Australia with annual surveys of 80 locations in 2016 and 2017. The emergence and spread of complex resistance haplotypes following a well-documented recent introduction of azoles into Australian farming practices demonstrates rapid resistance evolution in the agricultural ecosystems.

This abstract was presented orally at 12:20 on Wednesday 22 May 2019
Dublin, May 2019

**ABS93600**

**The impact of barley variety rotation, mixtures, and intercropping on leaf disease and silage production**

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Western Canadian barley silage producers, whether they are meeting on-farm needs or local market opportunities, will often look at continuous barley production, which leads to productivity issues related to leaf disease development. Although fungicides can be used, they represent an added input cost for silage producers. The objective of the current study was to determine the effects of monocultures, mixtures, intercropping and rotational diversity on crop health and productivity in a cereal silage production system. Three year rotational treatments were established in 2008 at Lacombe, Alberta with a final combined comparison for 2010, 2013 and 2016. Treatments included: continuous barley, same variety; a mixture of the same three barley varieties each year; an intercrop of barley, oat, and spring triticale with the same or different crop varieties each year; and an intercrop of barley, oat, and winter triticale with the same or different crop varieties each year. In 2010, 2013 and 2016, all treatments had the six-row barley variety Sundre. Leaf disease severity on Sundre was highest for continuous Sundre, and lowest for mixtures or intercrops with different varieties. Silage yields were lowest for continuous Sundre, highest for the intercropping treatments with the same or different varieties each year, and intermediate for barley mixtures where the variety components changed each year. Results suggest that adding diversity in crop types and/or barley genetics may reduce leaf disease and improve silage productivity.
Mechanism of multidrug resistance and risk assessment towards fungicides in *Zymoseptoria tritici*

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Multidrug resistance, or MDR, is characterized in *Zymoseptoria tritici* by low levels of resistance to DMIs, QoIs and SDHIs. The associated mechanism is the overexpression of the membrane transporter MFS1, inducing a more effective efflux of these fungicides outside the fungal cell. Three insertions of nucleotide sequences (types I to III), varying according to their size and position in the promoter of mfs1, are responsible for the overexpression of MFS1 and therefore of the MDR phenotype. This resistance mechanism is independent from the more frequent fungicide target modification mechanism, and in *Z. tritici*, sexual reproduction allows the combination of these mechanisms. Crosses between isolates carrying either MDR or target site resistance revealed semi-isogenic progeny that we used to characterize resistance. We thus show a significant increase in the levels of resistance to DMIs and SDHIs, compatible with losses in efficacy in practice, when the two mechanisms are combined in the same isolates. Moreover, the in vitro and in planta growth of isolates bearing these resistance alleles, solo or combined, doesn't seem to be affected, suggesting the maintenance of a fitness identical to that of sensitive individuals or presenting only one resistance mechanism. Field trials, carried out by the “Performance network” in France between 2004 and 2017, identified the programs most likely to be involved in MDR selection. Finally, we discuss management measures to limit the selection of different resistance mechanisms.

This abstract was presented orally at 14:00 on Wednesday 22 May 2019
Impact of wheat cultivar mixtures on Zymoseptoria tritici evolution over the course of an annual epidemic: the case of the ongoing breakdown of Stb16q

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The ability of wheat cultivar mixtures to reduce Septoria tritici blotch progress has already been demonstrated. However, much less is known about their impact on the durability of resistance genes. To this end, we compared pathogen adaptation to resistance when deploying a cultivar carrying the Stb16q gene (currently at the outset of breakdown) in mixtures with a susceptible cultivar (in resistant seed number proportions of 25, 50 and 75%) and in pure stands in a micro-plot field trial. We monitored both epidemic dynamics and the changes in the proportion of virulent strains to Stb16q in Zymoseptoria tritici populations over a cropping season. Thirty isolates were collected at the beginning (February) and at the end (June) of the epidemic on each cultivar in each micro-plot. Seedling virulence assays were conducted in the greenhouse to assess the impact of the proportion of the resistant cultivar in the micro-plots on the evolution of virulence frequency. A significant reduction in disease severity was observed both on the susceptible and on the resistant cultivars in mixtures. In February, 13% of the isolates collected on the susceptible cultivar in pure stands were established virulent, confirming that Stb16q breakdown is in progress; as expected this frequency was not different in cultivar mixtures. In June, the frequency of virulent strains slightly decreased on the susceptible cultivar in pure stands, suggesting a reproductive fitness cost associated with virulence; this frequency significantly increased with the proportion of the resistant cultivar in the mixtures (19, 51 and 79% of the isolates collected on the susceptible cultivar in mixtures with 25, 50, 75% of resistant cultivar, respectively). Cultivar mixtures thus reduced disease severity (protective effect of the resistant cultivar) but they contribute to the resistance breakdown by increasing the spread of matching virulence in local populations (selective effect of the resistant cultivar).

This abstract was presented orally at 14:20 on Wednesday 22 May 2019
Assessing the risk of resistance selection towards QiI fungicides in Zymoseptoria tritici

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Zymoseptoria tritici is a phytopathogenic fungus responsible for Septoria tritici blotch (STB) on wheat. During high disease pressure years, yield losses can reach 50%, making STB the first threat for wheat production in Europe. Chemical control remains the most effective way to control STB in the field but generalized resistance towards anti-microtubule benzimidazoles, DMIs and QoIs as well as the first reported cases of SDHI resistance, stress the need for greater diversity in efficient modes of action and improved resistance management. In this very challenging context, the launch of fenpicoxamid is highly anticipated since it provides a novel mode of action in the cereal market, acting at the Qi site in complex III of the mitochondrial respiratory chain, and is unaffected by target site-based resistance to other chemistries. Introduction of fenpicoxamid will be accompanied by implementation of anti-resistance strategies to preserve its long-term effectiveness. Our project aims to assess the risk of resistance towards fenpicoxamid in a realistic manner before its introduction in the field. We have developed an experimental evolution protocol to isolate strains with reduced sensitivity to complex III inhibitors in the laboratory with the goal of mimicking potential field selection conditions as closely as possible. It is hoped that insights into potential resistance mechanisms may be useful in optimizing anti-resistance strategies in the field.

This abstract was presented orally at 14:40 on Wednesday 22 May 2019
Diverse genetic resistance is the most effective and environmental friendly measure to manage any disease, therefore, multiple resistance is an obvious approach to prevent/reduce any possible genetic vulnerability in wheat. *Pyrenophora tritici-repentis* (PTR); *Zymoseptoria tritici* (STB); yellow and leaf rust frequently occur together and thereby present important resistance breeding targets. STB exhibits differential pathogenicity towards durum and bread wheat and also is highly variable genetically, suggesting that most resistance genes will be short-lived and a continual need to identify new strategies for effective disease management. Quantitative virulence in STB matches the quantitative nature of resistance observed in the host, but qualitative gene-for-gene interactions have also been reported for specific isolate–cultivar combinations. Resistance to STB is mainly quantitative, with 13 resistance genes identified and mapped to date, although the function of most of these genes is, as yet, unknown. Eight races of PTR are defined by their ability or inability to induce distinct symptoms (necrosis/chlorosis) caused by necrotrophic effectors (host-selective toxins), with ‘basic’ races producing only a single toxin and ‘composite’ races producing multiple toxins. Three dominant and independently inherited genes were shown to control sensitivity to each of the host-selective toxins, with one gene for each toxin. Although biotechnological approaches have much potential and will be very useful in specific situations, particularly in genetic analyses in the laboratory, they probably will be of less use in classical plant breeding programs due to the cost and complexity of the assays. This again brings us back to the basics: accurate phenotypic scoring of host reactions is still the most time-consuming and difficult part of the process, yet it is essential for future progress.
Variety mixtures: a promising strategy to improve STB disease management

Septoria tritici blotch (STB) caused by *Zymoseptoria tritici* is the most important foliar disease of durum wheat in Tunisia. Popular old variety Karim occupying 60% of cultivated area, is highly susceptible to STB and yield losses easily reach up to 40%. Disease management mainly relies on the use of fungicides and resistant varieties. Nevertheless, variety mixtures may provide more sustainable disease management.

The objective of this study was to test the effect of added resistant variety in mixtures with Karim for their capacity to cope with disease and to improve yield. Two resistant durum wheat varieties, Salim and Monastir, and the susceptible variety Karim, were assessed in pure stands and different mixtures at the CRP Wheat Septoria Phenotyping Platform at Kodia station (Tunisia). STB levels were measured twice: on F-1 leaves and a month later on Flag leaves. Leaves were collected at random, incidence was assessed visually on the collected leaves, and diseased leaves were analyzed using automated image analysis. Severity was measured as the percentage of leaf area covered by lesions and the density of pycnidia within lesions. Yield performance of these mixtures was measured by calculating thousand kernel weight (TKW) and grain yield (kg/ha).

Results suggest that STB is efficiently controlled by adding a proportion of resistant variety to a susceptible variety. Karim showed the highest disease levels, but adding only 25% of resistant cultivar resulted in significant decrease of the disease to a level comparable to pure stands of the resistant varieties. Moreover, treatments with 25% resistance had also significantly higher TKW and grain yield than what expected from linear relationship.

The analysis confirms that adding 25% of resistant component into mixture with susceptible variety suppresses the epidemics and increases yield. Hence, variety mixtures are a promising and efficient strategy to improve the disease management.

Keywords: variety mixtures, *Zymoseptoria tritici*, durum wheat, sustainable disease management

This abstract was presented orally at 15:20 on Wednesday 22 May 2019.
Dublin, May 2019

ABS33482

Airborne inoculum, fruiting body and genetic structure monitoring provides evidence for active sexual reproduction of Zymoseptoria tritici on durum wheat

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Septoria tritici blotch (STB) caused by the heterothallic ascomycete Zymoseptoria tritici is currently one of the most devastating diseases of wheat worldwide. The extent of sexual reproduction of this pathogen is well documented on bread wheat, but not on durum wheat. The objective of the present study was to quantify the potential of Z. tritici sexual reproduction on durum wheat in the Tunisian environment. The assessment was undertaken using a triple approach combining fruiting body assessment, ascospore trapping and population genetic analyses. The results highlighted the formation of pseudothecia on leaves and stubble from the autumn until the end of the growing season. Likewise, qPCR monitoring highlighted a constant release of Z. tritici airborne inoculum during the wheat-growing season, with a peak of production at the end of the season. Genetic investigations using microsatellites revealed high levels of genic and genotypic diversities, an equal distribution of mating types and a lack of genetic clustering within and between growing seasons. Taken together, these findings agree in suggesting strong potential for sexual reproduction on durum wheat in Tunisia for Z. tritici. Interestingly, the frequencies of detection and the quantities of trapped spores in Tunisia under low STB pressure on durum wheat are comparable to those previously found under severe STB pressure on bread wheat in Western Europe. This suggests that Z. tritici sexual reproduction in the Tunisian environment should occur to the same extent than Western Europe or could even be more important in epidemics. Such findings could explain the recurrence of strong STB epidemics in Tunisia and could be a valuable factor to take into consideration in STB management programmes on durum wheat.

This abstract was presented orally at 15:40 on Wednesday 22 May 2019
Understanding the impact of alternation on resistance evolution by the mean of experimental evolution: the case of *Zymoseptoria tritici*, the causal agent of septoria leaf blotch

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Enhancing the sustainability of the fungicide active ingredients is one of the challenges that need to be faced in crop protection. Though, predicting resistance evolution and the efficacy of anti-resistance strategies for a given resistance case could help risk assessment.

*Zymoseptoria tritici* is the causal agent of septoria leaf blotch and is the most detrimental fungal pathogen of wheat. In Europe, it has evolved fungicide resistance in the field to all unisite inhibitors, leading to variable efficacy losses depending on the modes of action and resistance frequency. This highly adaptive pathogen is then a challenging candidate for which preventive resistance management needs improvement.

This study aims to analyse the impact of modes of action alternation on resistance evolution using experimental evolution. Indeed, experimental evolution tends to accelerate in lab-standardized and miniaturized conditions the selection observed *in natura* and may help to the quick identification of operational drivers of this strategy. We expect that alternation may slow down the rate of resistance, compared to straight selection, that there is a relationship between the frequency of alternation and the rates and outcomes of resistance evolution, and that the heterogeneous selection pressure over time created by alternation may influence levels and mechanisms of resistance. Independent lines resistant to three fungicides (benzovindiflupyr, carbendazim and prothioconazole-desthio) were selected from the ancestral WT strain IPO-323, according to different alternation regimes. Resistance and fitness were measured in evolved lines. The comparison of resistance dynamics in between evolved lines was used to characterize the sustainability of the selection regimes.

This abstract was presented orally at 17:00 on Wednesday 22 May 2019
Every year crops come under attack from cereal pathogens, which drastically reduces their yield and economic viability. Disease control is often achieved through the application of fungicides, however, over use of fungicides leads to the occurrence of fungicide resistance mutations in pathogen populations. This can be seen in the cases of resistant populations of *Zymoseptoria tritici* that can be seen across Europe. Fungicide resistance is a major threat to pathogen control worldwide and has the potential to cause total crop losses and huge economic damage if left unchecked. Therefore, monitoring fungicide resistant mutations within fungal populations is crucial to inform management strategies and prolong the efficacy of fungicide treatments. Historically, the method used to analyse field isolates for fungicide resistance mutations is largely phenotype driven. This is a lengthy and labour-intensive process where each fungal pathogen is isolated and cultured using a species-specific method. To address this problem, we have developed a method capable of processing samples from 6 fungal species (Wheat powdery mildew, Yellow Rust, Brown Rust, Septoria Leaf Blotch, Net Blotch and Ramularia Leaf Spot) simultaneously across several fungicide target genes that would accelerate the identification of fungicide resistant pathogen strains within the field. Using this novel method, fungicide resistance monitoring can be more cost-effective and is capable of being fully automated in the future to reduce turn-around time. This knowledge can be used to develop tailored chemical control programmes maximising effectiveness while reducing chemical input. We have used our newly developed method to analyse infected leaf samples collected from field trial sites from Europe across multiple growing seasons. The availability of this rapid pipeline will transform the field of fungicide resistance monitoring by allowing high-throughput, large scale analysis of cereal pathogens.
One of the major obstacles to durable control of *Zymoseptoria tritici* has been its high adaptive potential, as shown through the evolution of resistance to multiple classes of fungicides as well as overcoming multiple host resistance genes.

We can therefore predict, for any new fungicide, that there is a risk of resistance evolving; but can the precise nature of resistance be predicted in advance? Predicting the form of resistance would allow diagnostics to be developed pre-emptively, and resistance management guidelines to take account of likely resistance levels and cross-resistance patterns. At a more fundamental level, this system can be used as a model to tell us about the predictability or otherwise of evolution in general. Is the evolutionary outcome highly dependent on the earlier steps, making it contingent on historical factors such as which fungicides were used when and which mutations appeared first? Or do functional constraints, such as the need for the fungicide target site to retain its original enzymatic activity, limit the evolutionary options such that any different pathways will soon converge on the same destination?

We make use of the functional genetic tools available in *Z. tritici* to investigate the functional constraints on target site evolution, constructing alternative haplotypes to test the viability of alternative evolutionary pathways. We also carry out experimental evolution, simulating different scenarios of fungicide use from different genetic starting points. We have also evaluated, in hindsight, the predictability of evolution in older fungicide classes for which resistance is already widespread, determining which factors are important for future predictions.
Vegetative hyphal fusions (VHFs) allow the formation of interconnected hyphal networks in filamentous fungi, which favors the distribution of nutrients and signals within the fungal colony. Contrary to cell fusions during sexual reproduction that requires different mating types, VHFs occur between genetically identical individuals. The best characterized gene required for VHFs is the soft ($S_o$) gene, which was first identified in the fungus *Neurospora crassa* and its deletion results in a complete absence of VHFs and shortened aerial hyphae. $S_o$ is highly conserved among Ascomycetes fungi, and it has been shown to be essential for hyphal fusion and pathogenicity in *Fusarium oxysporum* and *Alternaria brassicicola*. However, the connection between hyphal fusion and pathogenicity is still unclear. Here we have investigated VHFs and their role in virulence in the wheat pathogen *Zymoseptoria tritici*. We used strains expressing different cytoplasmic fluorescent proteins to detect cytoplasm mixing after cell fusion events, and we found that hyphal fusions are frequent in this pathogen both *in vitro* and *in planta*. We identified the $S_o$ gene in the *Z. tritici* genome and obtained a deletion mutant ($\Delta S_o Z_t$). $\Delta S_o Z_t$ shows a complete absence of hyphal fusions, but the fusion ability is recovered when the mutant is complemented with the $S_o Z_t$ gene. The disease progression in $\Delta S_o Z_t$ is similar to wild type, but pycnidia are completely absent in the mutant. Despite the absence of pycnidia, confocal microscopy and qPCR measurements during leave infection showed an earlier increase of fungal biomass in $\Delta S_o Z_t$. In conclusion, we show that hyphal fusions are not required for host invasion and host damage in *Z. tritici*, but they are essential for pycnidia formation.

This abstract was presented orally at 09:40 on Thursday 23 May 2019
Insertional mutagenesis using TC1-mariner transposon impala is influenced by chromatin modifications in the wheat fungal pathogen Zymoseptoria tritici

A novel tool for insertional mutagenesis in Zymoseptoria tritici was developed using fungal TC1-mariner transposon impala. ATMT excision vectors containing an autonomous copy of impala inserted in the promoter of A. nidulans nitrate reductase gene were constructed, and introduced in a Z. tritici Nia1- mutant. We selected a transformant with an impala vector inserted in chromosome 1. impala excision events were selected by recovering nitrate-utilizing revertants. Most revertants (80%) had a single copy of impala inserted at a new genomic location. impala was inserted in all core chromosomes without “hot spots”, but displayed a preference for chromosome 1 (40% of insertions). This bias is likely due to preferential insertion of impala on the same chromosome as donor copy. impala was rarely inserted in accessory chromosomes (1/10 of expected insertions). At the gene level, impala preferentially inserted near transcriptional start sites (TSS, 72%) of expressed genes. Since accessory chromosomes and transposons are characterized by their enrichment in repressive chromatin marks, we hypothesized that impala insertion pattern could be influenced by chromatin modifications. Z. tritici histone deacetylases were inhibited with trichostatin A (TSA) to open chromosomal regions with hypo-acetylated histone repressive marks. TSA increased impala excision rate (2-fold), as well as the frequency of insertions in native transposons (5-fold). In addition, TSA abolished the bias toward insertions in chromosome 1. However, TSA did not increase the insertion rate of impala in accessory chromosomes. These experiments showed that inhibition of histone deacetylases modified impala insertion pattern, suggesting that its insertion is dependent on chromatin status of its target site. Overall, impala is active in Z. tritici. It integration bias toward promoter/TSS of expressed genes makes it suitable for insertional mutagenesis.

This abstract was presented orally at 10:00 on Thursday 23 May 2019
Development and Application of a novel genome editing technique using CRISPR/Cas9 in necrotrophic pathogens

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The significant threat that necrotrophic pathogens pose to global wheat production has driven an increased interest in better understanding the genetic background of these fungi. Early genetic studies in these pathogens were hampered by a lack of efficient gene targeting techniques. CRISPR/Cas9, a new genome-editing tool, has revolutionized reverse genetics by providing an efficient method for targeted mutations. This technique deploys Cas9 endonuclease and a 20bp sequence, gRNA which identifies a 3bp sequence, protospacer adjacent motif in genome, resulting in binding of Cas9gRNA complex with target gene where Cas9 initiates gene editing.

Despite the effectiveness of CRISPR/Cas9 tool, its application has only been limited to a few model fungal strains. Therefore, we tested the efficacy of CRISPR/Cas9 approach in two necrotrophic fungi; Zymoseptoria tritici and Parastagonospora nodorum. We studied the delivery of CRISPR/Cas9 in the form of a preassembled protein-RNA complex, Cas9-RNP, for initiating targeted editing of marker genes. The RNP technique is a preferred approach for Cas9 delivery as the protein complex is quickly degraded by protein degradation pathways once inside the cell minimising off-target mutations.

We demonstrate that transformation of the RNP complex with a selectable marker harbouring flanking DNA homologous to target resulted in homologous recombination efficiencies exceeding 70% in P. nodorum. We further demonstrated that homologous recombination was effective, albeit at lower efficiencies, when using 50bp flanks. In contrast, we were unable to generate any CRISPR/Cas9 mutants in Z. tritici using the RNP approach. We further attempted genome editing by constitutively expressing Cas9 within Z. tritici but again was unsuccessful suggesting that the genome editing approach is ineffective in Septoria tritici blotch pathogen. These data highlight the significant potential that CRISPR/Cas9 has in improving reverse genetics in plant pathogenic fungi but also that its efficacy is dependent on the targeted pathogen.

This abstract was presented orally at 10:20 on Thursday 23 May 2019
Septoria tritici blotch (STB), caused by *Zymoseptoria tritici*, is the most destructive foliar disease of wheat. Optimal chemical control can be challenging due to the widespread fungicide resistance in the fungal pathogen populations. Thus, improving varietal resistance is the most sustainable and economical method for future STB disease control. Chloroplast photoprotection plays a critical role in plant responses to environmental stress. In this work, we aimed to determine the role of photoprotection in the early disease response and resistance to STB. Two winter wheat cultivars that differ in photoprotective responses to STB were spray-inoculated with *Z. tritici* at growth stage 19. Components of photoprotection including non-photochemical quenching (NPQ) of chlorophyll fluorescence, carotenoids, reactive oxygen species (ROS), and the expression of genes implicated in ROS detoxification were quantified at 0, 8 and 24 hours post inoculation (hpi). Early defence responses to *Z. tritici* at 8 and 24 hpi were associated with slow relaxing (qI) NPQ and significant upregulation of genes encoding ROS scavenging specific to the chloroplast and the accumulation of carotenoids. Our results show that photoprotective mechanisms originating in the chloroplast are crucial in plant defence against *Z. tritici*. Further elucidation of the underlying molecular and genetic mechanisms can facilitate breeding of new genotypes with improved durable resistance to STB as well as to other important foliar crop pathogens.

This abstract was presented orally at 11:40 on Thursday 23 May 2019
Fungal plant pathogens have a large repertoire of genes encoding putative effectors, which are presumed to aid infection. A large fraction of these genes is expressed only at low levels during in vitro growth but concertedly upregulated at specific stages of the infection. This tight regulation in time and space is postulated to avoid negative effects associated with mis-expression of effector genes, such as recognition by the host, autotoxicity or misuse of resources. Like in other fungal plant pathogens, many putative effector genes of *Zymoseptoria tritici* are located in transposable element-rich regions of the genome. These regions are usually heterochromatic and the expression of effector genes residing therein has therefore been hypothesized to be controlled by epigenetic mechanisms. In this work, we aim to determine the role of epigenetics in effector gene regulation in *Z. tritici*. We used reporter genes to monitor transcriptional activity at specific effector loci and showed that several effector genes are silenced in the absence of the host. During host colonization, de-silencing of effector genes occurs mostly when the fungus enters the host. Both increasing the levels of histone acetylation and abolishing histone H3 lysine 27 methylation impaired silencing, indicating that chromatin de-condensation plays a crucial role for effector gene activation. This research will contribute to our understanding on how the activity of the promoter and the genomic location enable fast induction of effector genes, while preventing the negative effects associated with mis-regulation of effector genes.

This abstract was presented orally at 12:00 on Thursday 23 May 2019
Investigating the role of taxonomically restricted genes and their potentially antagonistic role in combatting secreted fungal effectors

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Effector triggered immunity and effector triggered defence are crucial in combatting pathogens specialised at infecting a particular host. In the case of the Wheat/Z. tritici interaction, a 10,000-year-old conflict has led to considerable armaments being developed on both sides. On the fungal side, effector proteins are secreted into the host capable of evading detection, suppressing defences and altering responses are employed. In response to these threats, plant host receptors and small secreted proteins capable of detecting and countering these effectors are employed. A microarray focused on the latent phase response of a resistant (cv. Stigg) and susceptible (cv. Gallant) to Z. tritici infection was mined for taxonomically restricted genes (TRGs) within the Poaceae. From this analysis, we identified three TRGs that were significantly upregulated by Z. tritici. We silenced these three genes using virus induced gene silencing and observed an increase in susceptibility to STB in both cultivars. Most interesting was one gene, ‘TRG7’ that when silenced in cv. Stigg results in a breakdown of Stigg’s resistance. To test for interactions with secreted Z. tritici effectors, these TRGs were screened with a battery of small secreted proteins from Z. tritici using a yeast 2 hybrid system. Based on this screen, it appears that all three of these TRGs interact directly with at least one small secreted effector protein candidate of Z. tritici and could potentially be involved in combatting the secreted fungal effectors of Z. tritici.
Barley yield can be reduced by Ramularia Leaf Spot (RLS) disease which is caused by the fungus *Ramularia collo-cygni* (*Rcc*). *Rcc* can be present in barley throughout the life of the plant, but disease symptoms don’t usually occur in the field until after flowering. Although there have been significant advances in our understanding of the barley – *Rcc* relationship in recent years, the nature of the interactions between plant and fungus during the long period before disease symptoms appear is still not well understood. More specifically it is not known to what extent photosynthesis and plant growth is restricted during the asymptomatic phase of pathogen growth. My project aims to answer whether infection with *Rcc* affects barley leaf physiology during the period before visible disease symptoms appear, and if so whether there is any indication that effects from this stage of infection with *Rcc* could contribute to yield loss. I have used techniques such as qPCR, infra-red gas analysis, and chlorophyll fluorescence imaging to determine the relationship between the amount of pathogen infection, the severity of visible symptoms, and the rates of leaf photosynthesis and net carbon assimilation in both the symptomless phase and symptom expressing phases of pathogen growth. My results so far show that there is no indication of significant damage to the photosynthetic apparatus before symptom appearance, making it unlikely that the asymptomatic stage of infection is a major contributor to yield loss, however, once symptoms appear effects on photosynthetic performance extend beyond the lesion area.

This abstract was presented orally at 12:40 on Thursday 23 May 2019
Tan spot, caused by *Pyrenophora tritici-repentis*, is a serious disease of wheat, in Algeria for a long time. A collection of isolates sampled from several wheat growing areas in Algeria, for several seasons have been studied. They showed a wide morphologically variation; this includes cultures characters, mycelia grow, sporulation potential and conidia measurements. Races population structure analysis, using a differential host set among which, both bread and durum wheat were included, was performed. Races 1, 4, 5, 6, 7, and 8 were found and a new virulence pattern was discovered. Isolates with this new pattern induced necrosis in durum wheat but failed to induce any disease in the common wheat genotypes (Glenlea, 6B662 and, 6B665) in the differential set. Molecular characterization showed that isolates of this virulence pattern, harbor ToxA and ToxB genes; sequencing showed that their coding regions are identical to those of functional virulence genes. Amplification of ToxA and ToxB virulence genes showed that the genome of isolates sampled from Algerian field harbored both genes, their distribution through growing wheat areas have been established. Otherwise, fluorescent Amplified Fragment Length Polymorphism (AFLP), revealed high genetic diversity. Cluster analysis of molecular data showed that clustering of the isolates was independent of their race classification, geographic origin, or host plant. However, one isolate that showed a new virulence pattern was clearly distinguished from the rest of the population studied. Using resistant varieties still the best way to overcoming this disease, a seedling reaction of commercial wheat varieties evaluated in controlled conditions, showed that few varieties of durum wheat have an appreciable level of résistance.
Screening of small secreted proteins of *Z. tritici* reveals a candidate protein that interacts with host ubiquitin system

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Septoria Tritici Blotch, caused by the ascomycete fungus *Zymoseptoria tritici*, is a major threat to wheat production worldwide. The *Z. tritici* genome encodes for many small secreted proteins (ZtSSP) that likely play a key role in the successful colonisation of host tissues. In this study, we screened thirty of these ZtSSP for the induction of cell death in the non-host model plant *Nicotiana benthamiana*. We identified five novel candidates that resulted in the rapid accumulation of H$_2$O$_2$ and cell death. Transient overexpression of these five candidates also resulted in the upregulation of defense marker genes in *N. benthamiana*. These five candidates were further tested for cell death induction in the host wheat. However, our assay showed that none of these induce cell death in wheat, which might suggest host specificity of ZtSSP function and lack of recognition in the host. Additionally, yeast two-hybrid assay using one of the cell death-inducing candidate ZtSSP2 showed that it interacts with wheat E3 ubiquitin ligase in yeast and *in planta*. Expression analysis of wheat ubiquitin ligase suggests that wheat infected *Z. tritici* plants showed significant downregulation of TaE3UBQ expression at specific time points (4 dpi and 21 dpi) suggesting its potential role in regulating defense signaling during the wheat-*Z. tritici* interaction.

This abstract was presented orally at 14:20 on Thursday 23 May 2019
Genome wide association in *Parastagonospora nodorum* identifies novel necrotrophic effectors with unique characteristics

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*Parastagonospora nodorum*, employs necrotrophic effector (NE) proteins to facilitate disease. These NEs target host sensitivity genes to initiate defense response pathways including programmed cell death (PCD), with the result being to the advantage of the pathogen rather than the host. Nine NE-sensitivity gene interactions have been observed within this pathosystem, however, genes encoding *SnTox2* and *SnTox6* remained undiscovered. Genome resequencing of 198 *P. nodorum* isolates and phenotyping of host differential lines enabled an association mapping (AM) approach to identify novel NEs. AM using 322,613 polymorphisms identified loci associated with virulence on differential lines BG223 (*Snn2*) and ITM37 (*Snn6*). The putative *SnTox2* and *SnTox6* marker trait associations mapped to the same locus on chromosome 14, indicating that *Snn2* and *Snn6* were targeted by the same NE. Disruption of the top candidate gene at the *SnTox2*/*6* locus in isolate Sn4 resulted in the loss of virulence on both *Snn2* and *Snn6* differential lines, as well as failure to detect *Snn2* and *Snn6* QTL when inoculated onto the corresponding wheat mapping populations that segregate for *Snn2* and *Snn6*. Transformation of avirulent isolate Sn79-1087 with *SnTox2/6* resulted in virulence on lines harboring *Snn2* and *Snn6*, as well as detection of the *Snn2* and *Snn6* QTL. *SnTox2/6* is upregulated *in planta*, reaching peak expression at 24 hours post-inoculation. The results indicate *SnTox2/6* is a single NE which elicits PCD via detection by two non-homoeologous host targets.

This abstract was presented orally at 14:40 on Thursday 23 May 2019
Cloning of AvrStb9, a gene of Zymoseptoria tritici conferring avirulence on wheat cultivars carrying the Stb9 resistance gene

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The septoria leaf blotch disease is caused by the ascomycete fungus Zymoseptoria tritici. Nowadays, it is one of the most damaging diseases to wheats and triticale cultures in many regions of the world. The interaction between wheat and Z. tritici is complex because of its highly polygenic and mostly quantitative nature. Nevertheless, several wheat resistance genes, called Stb genes, correspond to the description of major resistance genes, i.e. qualitative phenotypic effect and involved in a gene-for-gene interaction with the fungus. So far, twenty-one qualitative Stb genes have been mapped in bread wheat. AvrStb6 was the first gene identified that confers avirulence to wheat cultivars carrying a known Stb gene, Stb6. Here, we report the cloning of AvrStb9 conferring avirulence to wheat cultivars carrying Stb9. AvrStb9 was identified by GWAS with Z. tritici isolates pathotyped on cultivar Soissons. The isolate IPO-09593 is avirulent while the reference isolate IPO-323 is virulent on Soissons. Replacement of the virulent allele with the AvrStb9 allele from IPO-09593 conferred an avirulence phenotype to transformed IPO-323 strains. Moreover, different recombination events in the AvrStb9 gene among the IPO-323 transformants allowed us to identify the protein domains involved in virulence. AvrStb9 encodes a large potentially secreted protein of unknown function. It is expressed in planta during the transition from the asymptomatic to the necrotrophic phases of infection. The resistance Stb9 was first reported in cultivars Courtot and Tonic. We identified Stb9 following a genome-wide association study (GWAS) on elite bread wheat cultivars, and confirmed its presence in Soissons by QTL mapping in a population Beaver/Soissons. The gene-for-gene relationship between AvrStb9 and Stb9 was shown by phenotyping Z. tritici transformants with either the virulent or the avirulent allele of AvrStb9 on near-isogenic lines carrying the resistant or susceptible allele at Stb9 in Courtot.

This abstract was presented orally at 15:00 on Thursday 23 May 2019
The symptomless latent phase of septoria tritici blotch (STB) disease remains elusive and under-characterised. Although the length of the latent phase has some bearing on resistance in wheat cultivars, the role of the latent phase in pathogenicity of the fungus remains largely unknown. Moderate resistance to STB in the variety ‘Stigg’ is hallmarked by a long (~36 days) latent phase, where after the disease progresses with the same vigour as in more susceptible varieties. The variety ‘Longbow’ is extremely susceptible to STB, exhibiting a classic latent phase (~12 days) followed by rapid progression of the disease. To extricate the molecular mechanisms behind Stigg’s exceptional resistance, we used time-course transcriptomics of Stigg (resistant) and Longbow (susceptible), inoculated with an aggressive Irish field isolate of Zymoseptoria tritici and sampled at four early timepoints post inoculation: 6 hours, 24 hours, 48 hours, and 96 hours.

As early as 6 hours post inoculation, the time the fungal spores start to germinate, 277 genes were up-regulated by the fungus – 163 in Longbow (S), 102 in Stigg (R), and 11 in both. Of the genes up-regulated in Longbow, the oxidation reduction process – a classic plant disease response – was the most represented biological process. Genes involved in oxidation reduction were also present in the up-regulated genes from Stigg, but the most common biological process in Stigg was protein phosphorylation – a category entirely missing from the susceptible variety. Protein kinases, responsible for phosphorylation, play a central role in signalling during pathogen recognition and the activation of plant defence mechanisms. By 24 hours post inoculation, there is some protein kinase activity in Longbow, suggesting a lag in pathogen detection in Longbow compared to Stigg.

At 24 hours, the up-regulated genes in Longbow are dominated by those involved in transcription and oxidation reduction. In Stigg however, transmembrane transport was the dominant biological process. Again, we see a time-delay in this family of genes, with only one up-regulated transporter protein at 24 hours in Longbow and one at 48 hours, although this is not the same gene.

These genetic responses are not in themselves unusual signatures of disease response, however the early expression of protein kinases and membrane transporters in Stigg may explain how this variety fends off pathogen attack for so much longer than more susceptible cultivars.
Long-term screening of winter wheat cultivars for Septoria nodorum blotch resistance

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Between 2009 and 2018, 2,161 winter wheat genotypes were tested as part of an ongoing US screening program to increase resistance to Septoria nodorum blotch (SNB), caused by Parastagonospora nodorum. The genotypes were mainly advanced experimental lines from 21 public and private breeding programs in the central and eastern US. Replicated trials were performed in 16 year*location environments (one or two environments per year) at public research stations in North Carolina, US. Plots were inoculated with P. nodorum-infected straw at about Zadoks growth stage 28. Irrigation was provided in some environments to ensure adequate disease development. Whole-plot disease ratings were on a 1-9 scale, with 1 the lowest and 9 the highest possible level of disease. While experimental lines were usually in the nursery fewer than 3 years, some commercial varieties were tested longer. To assess durability of quantitative resistance (QR) to SNB, a linear mixed model was fitted to data from the 19 genotypes that each appeared at least 7 years in the trials, plus one genotype that was tested 6 years and is known to possess quantitative resistance (QR) to several diseases. While mean levels of foliar and glume SNB increased significantly in the nursery over the period ($P < 0.002$), there was only weak evidence for variation in the rate at which foliar SNB changed on different varieties ($P = 0.06$), and no such evidence with regard to glume SNB, demonstrating durability of the QR in this germplasm. Of the 2,161 wheat genotypes phenotyped during that period, genotyping-by-sequencing data were available for 1,233. A mixed-model genome-wide association study was performed using data for those lines, and results of the study will be presented.

This abstract was presented orally at 16:40 on Thursday 23 May 2019
Ramularia leaf spot (RLS) is a destructive, late-season disease of barley caused by the fungus *Ramularia collo-cygni*. When it became commercially important in the late 1990s, the most RLS-susceptible barley varieties were those with *mlo* mildew-resistance\(^1\). The genetic association between *mlo* and RLS-susceptibility was confirmed but there was a substantial environmental influence on the effect of *mlo*\(^2\). Greater abiotic stress was found to increase RLS severity\(^3\).

There has been progress in breeding barley for RLS resistance\(^1,4\) but in field trials of a large set of spring barley varieties between 2010 and 2013, there was strong genotype-by-environment interaction (GxE) in RLS severity. Similarly, in UK Recommended List trials, varieties' scores at different sites have not always been consistent. The contribution of various factors to GxE was analysed in variety trial data from 2017. RLS is difficult to score accurately because it can be confused with several other syndromes\(^4\), and it was indeed found that different people do not score RLS in a comparable manner. This effect was not as strong as that of GxE, however, because there was substantial variation between variety scores obtained by one experienced person. Variation between sites is unlikely to result from variety-by-isolate specificity.

The use of sites where RLS is by far the predominant disease will enable breeders to continue advancing resistance by selecting select elite varieties which combine *mlo* for durable mildew resistance and minor genes dispersed throughout the genome for RLS resistance. Further control of RLS may be advanced by understanding the factors which caused the fungus to change from an insignificant endophyte to an aggressive pathogen in the 1990s.


\(^4\)Havis et al. 2015, *Phytopathology* 105:895-904
Precise phenotyping reveals novel loci for quantitative resistance to septoria tritici blotch in European winter wheat

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Accurate, high-throughput phenotyping for quantitative traits is the limiting factor for progress in plant breeding. We developed automated image analysis to measure quantitative resistance to septoria tritici blotch (STB), a globally important wheat disease, enabling identification of small chromosome intervals containing plausible candidate genes for STB resistance. 335 winter wheat cultivars were included in a replicated field experiment that experienced natural epidemic development by a highly diverse but fungicide-resistant pathogen population. More than 5.4 million automatically generated phenotypes were associated with 13,648 SNP markers to perform a GWAS. We identified 26 chromosome intervals explaining 1.9-10.6% of the variance associated with four resistance traits. Seventeen of the intervals were less than 5 Mbp in size and encoded only 173 genes, including many genes previously associated with disease resistance. Five intervals contained four or fewer genes, providing high priority targets for functional validation. Ten chromosome intervals were not previously associated with STB resistance. Our experiment illustrates how high-throughput automated phenotyping can accelerate breeding for quantitative disease resistance. The SNP markers associated with these chromosome intervals can be used to recombine different forms of quantitative STB resistance that are likely to be more durable than pyramids of major resistance genes.

This abstract was presented orally at 09:20 on Friday 24 May 2019
Accelerating the cloning of resistance genes against Septoria tritici blotch

Host resistance is one of the most sustainable ways to fight Septoria tritici blotch. However, a lack of cloned resistance genes limits breeding for resistant cultivars. Advanced genomic technologies have shaped the development of new approaches for rapid gene cloning. The “MutRenSeq” approach is a targeted resequencing method that was successfully developed for the discovery of plant disease resistance (R) genes encoding nucleotide binding and leucine-rich repeat (NLR) domains. However, not all genes conferring resistance to wheat diseases belong to the NLR family. Indeed, Stb6 and Stb16q, the only Septoria tritici blotch genes cloned thus far, encode receptor-like kinases (RLK). We therefore developed a strategy for targeted sequencing of this very large gene family. Using the most recent genomic resources from bread wheat and its relatives we identified ~45,000 RLK sequences corresponding to ~128 Mb, and developed a ~200,000 bait capture design. In a proof-of-concept experiment, we captured RLKs from seven EMS-derived loss-of-function mutants originating from the spring wheat Cadenza and quickly re-identified Stb6. This approach provides an opportunity to accelerate significantly the cloning of R genes encoded by RLKs to facilitate breeding for resistance to pathogens with apoplastic lifestyles, such as Zymoseptoria tritici or Stagonospora nodorum.

This abstract was presented orally at 09:40 on Friday 24 May 2019.
QTL mapping in a wheat MAGIC population reveals a consistent locus on chromosome 2A across locations and inoculation methods for leaf reactions to *Parastagonospora nodorum*

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The necrotrophic pathogen *Parastagonospora nodorum* is the causal agent of Septoria nodorum blotch (SNB). Compared to the other diseases in the wheat leaf blotch complex, Septoria tritici blotch and Tan spot, SNB is the most important wheat leaf blotch disease in Norway. The pathogen causes symptoms not only on leaves but also on glumes, which reduce both grain yield and grain quality. Former studies reported that the inheritance of resistance to leaf blotch and glume blotch is genetically different. Previous leaf blotch studies proved that *P. nodorum* interacts with host sensitivity loci via necrotrophic effectors (NEs) based on an inverse-gene-for-gene mechanism. However, the genetics underlying sensitivity to glume blotch still remains unknown. An interaction on wheat head partially regulated by gene involved in leaf blotch pathogenicity is one of the hypothesis suggested. In this study, an eight-parent winter wheat multiparent advanced generation intercross (MAGIC) population from the UK (‘NIAB Elite MAGIC’) was tested in field trials over four years for leaf blotch (4 trials in Norway, 2 trials in the UK) and two years for glume blotch (2 trials in Norway). Naturally infected straw was used as inoculum in Norway, while in the UK a spore suspension ($10^6 * 10^{-1}$) was applied twice during the growing season to inoculate wheat plants. Ten quantitative trait loci (QTLs) were identified across chromosomes 2A, 2D, 3A, 3B, 4A, 5D, 6A and 7D. Among these, a QTL on chromosome 2A was detected for both leaf and glume blotch. Haplotype analysis confirmed the consistency of this QTL across countries and inoculation methods for leaf blotch. However, the most susceptible haplotype for leaf blotch turned out to be the most resistant for glume blotch. Further investigation will be needed to dissect the mechanism behind leaf and glume blotch reactions caused by this QTL.

This abstract was presented orally at 10:00 on Friday 24 May 2019
Insights into the genetics of host-pathogen interactions for septoria nodorum blotch in Norwegian wheat fields and implications for resistance breeding

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Septoria nodorum blotch (SNB), caused by *Parastagonospora nodorum* is a major wheat disease in many humid and temperate production areas. In Norway, it is the most important of the leaf blotch diseases, although it often occurs together with septoria tritici blotch (caused by *Zymoseptoria tritici*) and tan spot (caused by *Pyrenophora tritici-repentis*). Insufficient resistance in current cultivars makes farmers highly dependent on fungicides for disease control. Resistance breeding based on phenotypic selection has been slow due to the quantitative nature of the resistance, confounding effects of plant height and phenology on disease symptoms and a poor understanding of the underlying genetic mechanisms. In recent years, substantial progress has been made in understanding SNB at the molecular level of both host and pathogen. The discovery of necrotrophic effectors (NEs) that cause susceptibility by interacting with corresponding host sensitivity genes pointed to the screening of breeding material for NE-sensitivities as a promising breeding strategy. On this background, research efforts were initiated in Norway in 2009 to characterize the local pathogen population, investigate the role of NEs in causing susceptibility under field conditions and map major resistance/susceptibility loci in relevant wheat germplasm. This research revealed a highly diverse locally adapted pathogen population with an effector-gene repertoire highly suited to match the prevalent *Tsn1* and *Snn3* sensitivity alleles in current wheat cultivars and breeding lines. A reliable field testing methodology based on naturally infected straw and mist irrigation was established, and has been used to screen germplasm and phenotype mapping populations and association mapping panels of both spring and winter wheat. Tox3 sensitivity was revealed as a major factor for field susceptibility to SNB in ToxA and Tox1 insensitive germplasm, and several promising quantitative trait loci (QTL) for field resistance have been detected in genome-wide association studies and QTL mapping in MAGIC populations.

This abstract was presented orally at 10:20 on Friday 24 May 2019
The fungus *Parastagonospora nodorum* causes septoria nodorum blotch of wheat. This disease is the outcome of multiple fungal necrotrophic effector-host sensitivity gene interactions that include ToxA-Tsn1, Tox1-Snn1 and Tox3-Snn3. Previous work demonstrated that the triple-knockout strain *P. nodorum* toxa13 maintained the ability to infect most modern bread wheat cultivars as effectively as the wildtype SN15. This suggests evidence of uncharacterised effector-host dominant susceptibility gene interactions or the lack of a resistance mechanism. To search for additional sensitivity genes, as well as putative resistance genes, a diversity panel of 295 historic wheat accessions from the N. I. Vavilov Institute of Plant Genetic Resources in Russia were chosen. This germplasm collection comprises of genetically diverse landraces and historic breeding lines registered from 1920 to 1990. Both *P. nodorum* SN15 and toxa13 were assayed on the Vavilov panel. SN15 was more virulent than toxa13. The subset of wheat lines insensitive to all three effectors showed significantly lower levels of disease when infected with SN15. However, the subset were no less susceptible to the toxa13 as the rest of the Vavilov collection. GWAS using SN15 assay detected quantitative trait loci (QTL) on chromosomes 1BS (Snn1), 2DS, 5AS, 5BS (Snn3), 3AL, 4AL, 4BS and 7AS. For toxa13 infection, a similar QTL was detected on 5AS, plus two additional QTL on 2DL and 7DL. Through historic temporal analysis, this study further revealed that plant breeders inadvertently selected for effector insensitivity from 1940 onwards. Outcomes from this study will help to identify accessions for development of bi-parental mapping populations to characterise resistance-associated alleles for subsequent introgression into modern bread wheat.
Genome wide association study of spot blotch at seedling and adult plant stages in India and Morocco

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Barley spot blotch caused by Cochliobolus sativus (Bipolaris sorokiniana) is one of the major constrains to barley production worldwide in warmer regions. The study was undertaken to identify and estimate effects of loci underlying quantitative resistance to spot blotch at seedling and adult plant stages. A panel of 261 barley genotypes (HI-AM) consisting of released cultivars from North and South America, Europe, Australia, advanced breeding lines, and landraces from ICARDA, was screened for assessing resistance to spot blotch. Seedling resistance screening was conducted using two most virulent isolates from Morocco (ICSB and SB54) while adult plant resistance was assessed at two hot spot locations in India (Faizabad and Varanasi) under artificial inoculation using a mixture of virulent isolates prevalent in Uttar Pradesh (India). Both GLM and MLM model were employed in Tassel using principal component analysis and Kinship Matrix as covariates. Genome wide association mapping indicated a total of 23 QTL at seedling stage (14 for isolate ICSB and 9 for isolate SB54), while 15 QTL were detected for adult plant resistance (6 at Faizabad and 9 at Varanasi). Common QTL at seedling stage and adult plant were found across all barley chromosomes. QTL detected explained together the 73.24% of the variance for seedling resistance to isolate ICSB and 49.26% for isolate SB54. QTL for adult plant explained together 38.32% and 44.09% at Faizabad and Varanasi, respectively. Several QTL identified in this study were also reported before in bi-parental and association mapping populations studies supporting our results. The promising QTL detected at both stages, once validated, can be used for MAS in spot blotch resistance breeding program globally.

This abstract was presented orally at 11:20 on Friday 24 May 2019
Ramularia leaf spot (RLS) is a newly important disease of barley that occurs late in the growing season and has been identified in all the temperate region of the world. The disease is caused by the Dothideomycete fungus *Ramularia collo-cygni* and RLS symptom development is thought to be associated with the release and action of toxic fungal secondary metabolites in the host. These metabolites called rubellins belong to the family of anthraquinone compounds. To date, six rubellin derivatives, named alphabetically, have been isolated from *R. collo-cygni* cultures with rubellins B and D being the most abundant in infected leaves. Rubellins are light-activated, non-host specific metabolites which have been shown to induce fatty acid peroxidation *in vitro* but their mode of action *in planta* has never been studied. Our study investigated the action of the most stable and soluble rubellin *in planta*, rubellin D, at the genetic level in the model plant *Arabidopsis thaliana*. Using infiltrations, we showed that rubellin-induced cell death, which appears phenotypically reminiscent of programmed cell death, involves the host unfolded protein response (UPR). This response is activated to avoid cell death resulting from an endoplasmic reticulum-related stress due to an accumulation of unfolded or misfolded protein and is partly mediated by the plant proteasome. In our study we also showed that rubellin-induced cell death is dependent on the host proteasome. Furthermore, we showed that the host salicylic acid pathway, which is known to activate the UPR upon pathogen recognition, is also involved in mediating rubellin-induced cell death. Taken together our data suggest that the host UPR plays a central role in rubellin-induced cell death.

This abstract was presented orally at 12:20 on Friday 24 May 2019.
Genomic and transcriptomic sequencing reveal a lack of diversity in European isolates of the causal agent of wheat leaf rust, *Puccinia triticina*

Brown rust, also known as leaf rust, is caused by the obligate biotroph basidiomycete *Puccinia triticina* and is the most common rust disease of wheat worldwide. Phenotypic data in the virulence profiles of UK brown rust isolates over the last 12 years has revealed four races of interest. Whilst the genetic diversity of *P. triticina* has been monitored across the world through the use of Simple Sequence Repeat (SSR) and Random Amplified Polymorphism DNA (RAPD) markers, genomic data has only recently become available, opening up new avenues of research. A novel pathogen surveillance technique, termed ‘field pathogenomics’, has been used to characterise the population diversity of the related wheat rust pathogen, *P. striiformis* f. sp. *tritici*. The method allows rapid detection of pathogen variants directly from infected wheat samples taken from the field through transcriptomic sequencing of infected material. In this study, we are using a genomic approach in combination with the field pathogenomics technique to characterise the European brown rust population and genetic diversity. This ongoing work involves genome sequencing of 44 UK brown rust isolates from the years 2006-2015 to characterise the UK brown rust population in the years prior to this project, accompanied by transcriptomic sequencing, using our field pathogenomics technique of ~40 European field isolates from each of the 2017 and 2018 growing seasons. Population genetic analysis of 112 brown rust isolates illustrated that they are all closely related with very little genetic diversity. The next step is to link the differences in the virulence profiles between brown rust isolates to any commonalities in polymorphisms in candidate effector genes for isolates with similar virulence profiles. This will contribute to our understanding of brown rust, and how isolates with such similar genetic diversity can present the different virulence profiles seen in the UK prior to this study.

This abstract was presented as a poster at Location 1
First evidence of moisture adaptation in *Zymoseptoria tritici*

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Temperature and moisture are critical micrometeorological factors affecting each epidemiological stage of foliar diseases. Several studies reported thermal adaptation of plant pathogens, including *Zymoseptoria tritici*, but no similar investigation has been carried out on the response and adaptation to moisture conditions. To detect if there is evidence of “moisture adaptation” in *Z. tritici*, we retained 48 genetically distinct isolates (already phenotyped for their thermal responses) from two populations collected in Ireland (IR) and Israel (ISR). These populations originate from contrasted climatic locations with respect to moisture conditions (annual air relative humidity - RH - of 85.1% vs 69.7%, respectively). We assayed the moisture response of these isolates on wheat seedlings during the earliest stages of infection (pycnidiospore germination, epiphytic hyphal growth and penetration). Lesion development was visually assessed under four post-inoculation RH regimes obtained by covering plants with polyethylene transparent bags for either 0, 1, 2 and 3 days post-inoculation (dpi). As expected, the longer the bagging time, i.e. the higher average RH, the more severe the symptoms were. A significant interaction between RH and population effects was established, suggesting that ISR was better adapted to lower humidity than IR. To characterize more finely this difference in phenotypic plasticity, moisture reaction norms describing the pattern of sporulating area at 14 and 17 dpi across the mean RH during the three days following inoculation were established for all isolates. Their sensitivity to RH was estimated by the slope of the linear relationship between mean RH and the percentage of sporulating area. The absence of genotypic differentiation for neutral microsatellite loci between both populations, established using 12 SSR markers, confirmed that this significant difference in RH sensitivity between IR and ISR can be interpreted as a signature of adaptation.

This abstract was presented as a poster at Location 2
Divergence of aggressiveness of *Zymoseptoria tritici* isolates on a susceptible wheat cultivar revealed by comparative genomics

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*postdoc*

*Zymoseptoria tritici* is a fungal wheat pathogen that causes Septoria tritici leaf blotch (STB), which is considered the most damaging disease of wheat in Europe. Phenotyping of multiple *Z. tritici* isolates on the wheat cultivar Longbow revealed divergence in aggressiveness on this particular wheat cultivar. This divergence in aggressiveness may be explained by large scale structural variations (presence or absence of chromosomes) or modifications at the nucleotide level within genes that encode for secreted proteins, so called effectors. Here, we aimed to determine the differential aggressiveness of these *Z. tritici* isolates on the wheat cultivar Longbow using comparative genomics and transcriptomics, while focusing on effector genes. Of all the phenotyped isolates, we observed that *Z. tritici* isolates, namely 553.11, S-Oak, and S-Cork were the most aggressive on wheat cultivar Longbow, whereas the remaining seven isolates showed moderate or reduced aggressiveness on this wheat cultivar. Comparative transcriptomics analysis revealed one effector gene that is significantly differentially expressed in the highly aggressive *Z. tritici* isolate 553.11 in comparison to the moderately aggressive *Z. tritici* isolates IPO323 and 560.11. Future functional analysis will be performed to confirm the contribution of this candidate effector gene to *Z. tritici* aggressiveness. Here, we determined genome-wide differences in a collection of *Z. tritici* isolates that have the differential capacity to infect the susceptible wheat cultivar Longbow, which will provide insights into *Z. tritici* aggressiveness.

This abstract was presented as a poster at Location 3
How mixed infection impacts the transmission of the wheat pathogen Z. tritici?

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Marcello Zala
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Bruce McDonald
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Natural infections are frequently produced by several strains that co-infect simultaneously the same host. Mixed infections affect the outcome of the infection, the epidemiology and transmissibility of the pathogen. However, so far, only few studies have investigated how mixed infections affect the infection dynamics and the evolution of virulence of fungal pathogens. This is also the case for the wheat fungal pathogen Zymoseptoria tritici. Z. tritici is so diverse that even in single lesions multiple strains co-exist and produce pycnidia. Our recent studies have shown that competition between strains leads to changes in the overall virulence and to a reduction in the expected number of pycnidia produced. In serial passage experiments, we demonstrated that one strain outcompeted the others. We observed that the outcome of the competition was dependent on the cultivar and not on the virulence phenotype of the strain. We suggest that the adaptation of each strain to a specific cultivar increased its competition capacity and provides an advantage to the strain to be successfully transmitted in mixed infections.

This abstract was presented as a poster at Location 4
Septoria nodorum blotch (SNB) caused by *Parastagonospora nodorum* is a major disease of wheat Western Australia and has been a major target for breeders for many years. We assembled a panel of 155 WA *P. nodorum* isolates collected over a 44-year period and compared them to 23 isolates from France and the USA using 28 SSR loci and to a large global population using 5 SSRs. The WA *P. nodorum* population could be divided into five groups with contrasting spatio-temporal distribution and different reproductive modes.

The majority of the WA isolates fell into two groups that were found throughout the collection area and had balanced mating type allele frequencies consistent with regular sexual reproduction. The other three groups were found in restricted locations, were transient and consisted of a single mating type consistent with asexual reproduction only.

The asexual groups coincided with the adoption of only a single or a small group of wheat cultivars. When introduced, these cultivars had high scores for SNB resistance but declined over subsequent seasons until replaced with new more resistant varieties. The asexual populations were more pathogenic on contemporary cultivars than the co-existing sexual populations.

The asexual populations was studied by comparing them with the sexual populations and a large group of overseas isolates and were found to be more closely related to overseas population consistent with regular invasion into WA despite the extensive biosecurity precautions.

The study has an important practical outcome as it can be used to define a rational method to select isolates to be used when testing the resistance of cultivars being trialled for release. To optimise this process, it is important to select both current most virulent isolates as well as examples of sexual populations. The implications of this study for this and related pathosystems will be discussed.

This abstract was presented as a poster at Location 5
High genetic diversity among Zymoseptoria tritici isolates from bread wheat in Northern Tunisia

ABS93193

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Septoria tritici blotch (SLB) is the most important foliar disease of durum wheat in Tunisia, hence previous genetic diversity analyses of the local Zymoseptoria tritici population mainly concentrated on isolates derived from Triticum durum. In this study, Z. tritici populations from bread wheat were investigated. A total of 184 single-pycnidial fungal isolates were recovered from naturally infected bread wheat leaves sampled in three main Tunisian growing areas (El Haouaria, Bizerte and Beja). The collection was assessed for mating type idiomorphs and genetic structure using 12 microsatellite markers at region and field scales. The results revealed high genetic diversity in the collected population. El Haouaria displayed the highest level of polymorphism (100%) and the highest Shannon diversity index (0.84). Such high diversity in El Haouaria is likely due to an active sexual reproduction of the pathogen, which is supported by the equal frequencies of the two mating types in that region. The study also revealed the absence of genetic variation in the Oued Zargua population in Beja region, suggesting that clonal pycnidiospores are the major source of primary infections. This study is the first population analysis of Z. tritici isolates from bread wheat in Tunisia and provides important insights into the genetic structure and epidemiology of STB that would be useful for an effective disease management.

This abstract was presented as a poster at Location 6
Zymoseptoria tritici populations are exposed to a wide range of temperatures both temporally over the epidemic period (from late autumn to early summer) and spatially across the worldwide wheat growing area. It is now well established that these variations in thermal conditions strongly affect epidemiological processes and dynamics. As such, it is of critical importance to quantify the scale and extent of thermal adaptation of Z. tritici populations. Previous research has established that there were signatures of seasonal selection dynamics and contrasted thermal sensitivity among world populations. However, the nature of phenotypic plasticity over the full range of encountered environmental conditions and the adaptation shifts along temperature gradients at both the individual and population levels remain unclear. We investigated the thermal responses of 18 Euro-Mediterranean (sub)populations (416 strains) sampled along: (i) seasonal gradients over an annual field epidemic (winter vs. spring populations); (ii) two French spatial gradients of increasing annual mean temperature and temperature range; (iii) Euro-Mediterranean climatic gradients representative of oceanic, continental and Mediterranean conditions. The full thermal responses of these strains were obtained experimentally using in vitro growth kinetics as a proxy for fitness when establishing thermal performance curves (TPC). Our results show that there is a pronounced intraspecific phenotypic plasticity and individual variability of TPC within local populations (e.g. co-existence of cold- and warm-adapted strains). In particular, we highlighted: (i) seasonal selective shifts in TPC with divergence in thermal optima of 3°C between winter and spring populations; (ii & iii) the existence of spatial thermal patterns with adaptation to local environments, but only at the Euro-Mediterranean scale. By expanding our understanding of the level of diversity in thermal responses of Z. tritici, this study provides a basis for exploring the effects of temperature on the adaptive potential of populations to their natural changing environment.
Race specific and race-non-specific interactions with barley have been previously reported for Bipolaris sorokiniana (BS). 322 single-spore isolates of BS collected in Uruguay from 2001 to 2010 were assessed for their interaction with 35 barley genotypes at the two-leaf stage under controlled conditions. The aims of the study were to characterize the Uruguayan population of BS, to determine if there are race-specific interactions, and to identify a manageable set of the most informative barley genotypes to characterize the pathogen. The quantitative interactions were analyzed with Hierarchical Clustering on Principal Components (HCPC), resulting in nine barley genotype clusters and six clusters of BS. Reducing the number of isolates to 147, based on redundant infection responses, resulted in eight barley genotype clusters and seven BS clusters to which none of the utilized barley genotypes tested were resistant. There were several BS clusters to which none of the tested barley genotypes were resistant, pointing to a serious lack of resistance sources in Uruguay. For screening purposes, 12 barley genotypes were selected based on their resistance profiles to identify isolates with differential interactions. These genotypes grouped into 11 clusters that interacted in a specific but quantitatively variable manner with the 147 isolates selected previously. The isolates grouped into eight clusters each representing different aggressiveness profiles. No specific pattern across years and collection locations were observed suggesting no population subdivision. Despite some clear isolate specific interactions, the overall quantitative interactions in this pathosystem make it unlikely that a universal differential set could be used to monitor BS diversity worldwide.
Leaf blotch caused by *Parastagonospora* and *Zymoseptoria* species are major diseases of cereals worldwide. In France, they are mainly caused by *Z. tritici* on bread wheat but only little information is available for durum wheat, barley, triticale and oat in France. Since *Z. tritici* and *Parastagonospora* species cause relatively similar symptoms on cereals, we needed to develop quantitative PCR (qPCR) methods to provide tools for the characterisation of the fungi complex involved in cereal leaf blotch in France.

Careful molecular characterisation and phylogenetic analyses of 90 strains isolated from wheat, barley, triticale and oat were performed and confirmed the presence of 4 *Parastagonospora* species or sub-species. Four real time species specific real time PCR assays were developed according to MIQE guidelines (The minimum information for publication of quantitative real-time PCR experiments). The specificity, the limits of detection and the limits of quantification were carefully assessed and fit the standard for validated tests. These assays and another specific to *Z. tritici* (previously developed) were used on symptomatic leaves collected in France to characterize the fungal species for several cereals. For wheat, as expected, *Z. tritici* was the most predominant species. For durum wheat, both *Z. tritici* and *P. nodorum* were significantly quantified. Interestingly, *P. nodorum*, *Z. tritici* and *P. avenae* f. sp. *triticea* were all quantified from triticale leaves samples. On the other hand, only *P. nodorum* barley biotype was significantly quantified from barley samples. This last result showed that the method is very specific of this biotype and seemed to confirm a host adaptation to barley for *P. nodorum*.

The tools developed in this study will be useful to characterize accurately the *Parastagonospora* species complex on cereals and be helpful to study epidemiology and host adaptation in order to provide the best integrated disease management.
Currently wheat production in western Europe is reliant on the application of plant protection products throughout the growing season. Fungicides account for approximately 25% of these applications, with septoria tritici blotch, caused by *Zymoseptoria tritici* the primary target. Unfortunately the evolutionary potential of *Z. tritici*, coupled with the high specific nature of current fungicide chemistries place the pathogen at a high risk of fungicide resistance development. Over the past decade the consequences of this have been observed at field level. In addition to fungicide resistance, changes in the registration of plant protection products within the EU will impact upon the availability of current and future chemistries. It is therefore imperative that both the potential development of resistance and subsequent spread is minimized. As part of the C-IPM ERA-NET, EUR-RES was established with in the EUROWHEAT platform to provide the basis from which to achieve this goal. Specifically the EURO-RES project aims to A) determine levels of resistance and the dynamics of resistance spread in partner countries B) determine the impacts of fungicide control strategies using the diversity in resistance levels throughout western Europe and C) make publicly available the research findings through the EUROWHEAT platform in such a manner that they can be readily utilised by extension services and growers.
Occurrence monitoring on *Parastagonospora* spp. and *Zymoseptoria tritici* of wheat and triticale under climatic conditions of Poland.

The most common wheat and triticale septoria leaf spot pathogens in eastern Europe are: *Mycosphaerella graminicola* (Fuckel) (anamorph: *Zymoseptoria tritici* (Desm.) and *Phaeosphaeria nodorum* (E. Müller) (anamorph: *Parastagonospora nodorum* (Berk.). *Phaeosphaeria avenaria* (G. F. Weber) (anamorph: *Parastagonospora avenae* (A. B. Frank) primarily was oat and rye based fungus. However, over time *P. avenae* special forms pathogenic to wheat and triticale have emerged. In 1980s the most common fungus among septoria diseases in eastern Europe appeared to be *P. nodorum*. Nevertheless, a shift in prevalence from *P. nodorum* to *Z. tritici* was observed over time in certain regions of Europe.

Spatial distribution and incidence frequency of pathogens is not constant, therefore monitoring of pathogens occurrence is the first necessary step to optimise breeding programs priorities. The aim of this study was to examine *Parastagonospora* spp. and *Z. tritici* severity and occurrence on wheat and triticale varieties to septoria leaf spot - LS and glume blotch - GB. Field experiments were established at 7 locations in various geographical regions of Poland. In each location a nursery consisted of 10 - winter wheat and 10 - winter triticale varieties was established to quantify LS and GB disease severity. Plants on plots were scored using 9 digit scale. Affected leaves and glumes samples were collected and analyzed using binocular and inverted microscope. The pycnidiospores were measured and identified to the species. The isolation frequency and diseases levels were quantified for each pathogen. Investigation showed *Z. tritici* predominance on wheat - 89%, while *P. nodorum* was the most frequently isolated from triticale - 62%. Incidence of *P. avenae* accounted for 1% of isolations on wheat and 4% on triticale. Varieties of wheat and triticale showing considerable levels of resistance to *P. nodorum* and *Z. tritici* were determined.

This abstract was presented as a poster at Location 11.
Like many other cereal foliar pathogens, *Zymoseptoria tritici* remains on crop residues during the intercropping season, where it reproduces sexually, with ascospores infecting seedlings at the beginning of the subsequent season. Despite the importance of residues as source of inoculum, few studies have examined the impact of the whole microbial community of this particular ecosystem on such kind of pathogens during their saprophytic/sexual stage. In this study we characterized the interactions between *Z. tritici* and the micro-organisms present on residues and assessed the impact of some factors (contact with soil, seasonality). To this end, residues of adult wheat plants preliminary inoculated with *Z. tritici* in greenhouse were left outdoor under different conditions, either with or without contact with soil. Non-inoculated residues were left in the same conditions as control. Residue sampling was performed in July (before contact with soil), in October, December, and February. This has been done two successive years. The analysis of the fungal and bacterial communities was carried out by metabarcoding approach with ITS and 16S primers. Overall, 420 residues samples were analyzed, with 15 replicates per condition. The impact of *Z. tritici* inoculation was significant for both fungal and bacterial communities. The main determinants of diversity were year for fungi, and soil contact for bacteria. Moreover, co-occurrence network analyses allowed to identify micro-organisms suspected to interact directly and indirectly with *Z. tritici*.
Detection of Ascospores of the Wheat Eyespot Pathogens *Oculimacula yallundae* and *O. acuformis* in Both Autumn and Spring in the Pacific Northwest USA

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*Oculimacula yallundae* (OY) and *O. acuformis* (OA) cause eyespot disease of wheat. These residue-inhabiting fungi produce conidia beginning in autumn that have been considered the primary inoculum in the U.S. Pacific Northwest (PNW). Apothecia of both species are also produced in commercial wheat fields in the PNW. Based on population genetic studies, it appears that both species are undergoing sexual reproduction in the PNW, but the role of ascospores in eyespot epidemiology is unknown since they are reportedly released in spring. A previous study conducted in inoculated field plots found ascospores of OY and OA were present in spring and autumn, with most in spring. This study sought to determine whether and when ascospores of OY and OA are present in non-inoculated commercial wheat fields. Ascospores were collected with Burkard 7-day recording volumetric spore traps at two locations (S=Spillman Farm; PP=Plant Pathology Farm; PC=Palouse Conservation Farm) each year during the 2014-15, 2015-16, 2016-17, and 2017-18 crop seasons near Pullman, WA. Spore-trap tapes were collected weekly and examined microscopically to confirm presence of ascospores and absence of conidia; DNA was extracted from the tapes and RT-PCR used to quantify DNA of OY and OA with species-specific primers. Ascospores of OY and OA were detected in all seasons at all locations, with the most collected in 2017-18 and 2015-16, and the least in 2014-15. More ascospores were detected in autumn than spring at three locations (S15, PP15, PC16), more in spring than autumn at three locations (S17, S18, PC18), and similar in autumn and spring at two locations (S16, PC17). More ascospores of OY than OA were detected in all years and locations. Based on these data, we conclude that ascospores of both pathogens are present during the primary infection period, which could explain the high genotypic diversity observed in previous studies.

This abstract was presented as a poster at Location 13.
Sensitivity of Algerian *Pyrenophora teres* population to QoI fungicides reveled by Pyrosequencing

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Net blotch of barley caused by *Pyrenophora teres* (Died.) Drecshler, is currently one of the most destructive diseases on Barley (*Hordeum vulgare* L.) crops in Algeria, fungicides treatments are mainly used to its control. A total of 230 mono-conidial isolates of this fungus were sampled in 2015/16 and 2016/17 seasons, from 17 Algerian provinces, in 58 distinct geographical localities where barley is cultivated. In order to identify fungicides resistant individuals, and prevent their extension in the pathogen population, isolates were assessed for resistance to Quinone outside inhibitors (QoI) fungicides. Resistance was screened using Pyrosequencing technology. F129L and G137R mitochondrial cytochrome *b* substitution associated with QoI resistance were looked for. Our results showed that all tested isolates were identified as QoI-sensitive, since all possessed the F129L and G137R wild-type alleles. According to these, *P. teres* Algerian population is considered sensitive toward QoIs, which is reassuring for farmers, and fungicides market in Algeria. This study is the first investigation related to mutations associated to QoI fungicides resistance in Algerian population of *P. teres*.

This abstract was presented as a poster at Location 14
RESIST – a multifaceted approach to manage the fungicide resistance of *Zymoseptoria tritici* in Belgian fields

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Owing to a lack of resistant varieties, fungicide application remains a key to prevent Septoria leaf blotch (STB) epidemics, caused by *Zymoseptoria tritici*. While continuous monitoring of fungicide resistance are performed annually in neighboring countries, no information was available regarding the current status of *Z. tritici* fungicide resistance in Belgium. In the framework of the RESIST project, a number of different approaches are undertaken to overcome this lack of knowledge.

A first effort aimed at readily determine the spatial distribution of resistance of *Z. tritici* in commercial fields from the south of Belgium using a population-based method developed by INRA-BIOGER. These results are complemented with results from microtiter plate assays performed on single-spore isolates. The relatively high frequency of Multidrug Resistance (MDR) strains and of strains highly resistant to demethylation inhibitors (DMI) was comparable to the situation observed in the North of France. As expected, populations were also virtually insensitive to quinone outside inhibitor (QoI) and methyl benzimidazole carbamate (MBC) fungicides, while resistance to succinate dehydrogenase inhibitors (SDHI) is still low. A drastic increase in resistance from a 2008-09 baseline population was also observed for multiple active ingredients. The use of allele-specific qPCRs on leaf samples and classic sequencing on pure strains confirmed the presence of multiple resistant genotypes. Moreover, microtiter plate assays are performed to evaluate the evolution of the fungicide sensitivity of *Z. tritici* field populations resulting from different fungicide programs commonly used in Belgium. To further investigate the extent of the fungicide-driven selection in the field, an *in vitro* procedure is being developed to replicate and study the effect of field treatments. A comprehensive review of these results as well as their implication on future management of *Z. tritici* resistance will be elaborated.

This abstract was presented as a poster at Location 15
Ramularia leaf spot of barley (RLS), caused by the fungal pathogen *Ramularia collo-cygni* has the potential to reduce Irish winter and spring barley varieties by up 15%. Fungicides are currently the only effective and reliable means of control. Unfortunately the disease often only exhibits symptoms post-anthesis meaning decisions on control, such as product choice and/or application rate, must be made in the absence of disease levels or risk indicators. In most instances growers will be risk adverse and routinely apply fungicides to control RLS. Leaf surface wetness during stem extension has been tentatively suggested as a risk indicator for RLS. To assess if this can be used as means of determining risk and subsequently if RLS treatments can be tailored to reflect this risk, spring barley field trials were conducted at two locations in Ireland during the 2016-2018 seasons. Fortunately each season presented different levels of both predicted risk and disease levels. Depending on risk levels as determined by minutes of leaf wetness during stem extension, it was possible to reliably reduce fungicide spend by either reducing the dose or the number of modes of action without adversely impacting control of RLS. Throughout the study the multisite fungicide chlorothalonil remained central to control of RLS. Whilst additional modes of action are anticipated in the near future, given the ability of *R. collo-cygni* to adapt and develop fungicide resistance to single site fungicides, it is anticipated that the potential withdrawal of chlorothalonil from use within the European Union will have adverse impacts on control of RLS in Irish barley crops.

This abstract was presented as a poster at Location 16
Mixtures of wheat cultivars can reduce disease propagation of septoria tritici blotch (STB) in the field. Growing together cultivars with contrasting levels of disease resistance provides disease protection for the most susceptible cultivar. Mixtures are generally constituted with cultivars of similar straw height. However, a contrast in plant height could have an impact on spore dispersal. For example, simulation studies showed that leaves from tall plants located above a short cultivar provide shelter from raindrops for diseased leaves at the bottom of the canopy, thus limiting the production of splash droplets carrying spores.

Though this « umbrella effect » has been identified using a modelling approach, little experimental data is available on STB epidemics in cultivar mixtures with contrasted plant height. The objective of our study was to assess disease propagation within two binary mixtures composed of a short susceptible cultivar (~80 cm) and a tall cultivar (~160 cm), either resistant or susceptible. Susceptible pure stands and mixtures were grown during two years in field plots. Disease severity and plant height were recorded at flowering stage.

Disease severity in susceptible plants was lower in both mixtures, composed of either resistant or susceptible tall cultivar, from 30 to 70% less than in pure stand, considering all plant leaves. Disease reduction in susceptible plants was enhanced in the mixture including a tall resistant cultivar. For example, no disease was observed during the first year on the top three leaves of susceptible plants within that mixture.

Our work suggests that mixtures of cultivars with contrasted plant height present a potential for enhancing STB severity reduction, possibly through physical mechanisms related to rain-splash dispersal. More extensive experimentations would be necessary to fully assess the relative contribution of the mechanisms leading to disease modulation in such mixtures.
A total of 100 single-spore strains of Zymoseptoria tritici were sampled in 2016 from northern France in order to conduct an inventory on resistance to demethylation inhibitor (DMI) fungicides in this region exposed to high disease pressure and frequent treatments with fungicides. Characterization using microsatellite markers showed that all strains were different multi-locus-genotypes. Sequencing of the Cyp51 gene highlighted 18 nucleotide changes (including single nucleotide polymorphism and indels), among which five were never reported in the literature. These changes collapsed the 100 strains into 23 distinct haplotypes. Other mechanisms involved in fungicide resistance, including multidrug resistance (MDR) and Cyp51 overexpression mechanisms, were also investigated for all strains using PCR. Among the 100 strains, only seven were MDR and only 14 overexpressed the Cyp51 gene. None of these strains combined both MDR and overexpression mechanisms. The strains were phenotyped against five DMI technical grades (epoxiconazole, prothioconazole, metconazole, tebuconazole, and prochloraz) using fungal cultures in microplates. Correlations between the genetic background of the strains (Cyp51 substitutions, overexpression, and MDR) and their sensitivity against the tested DMIs were statistically assessed. Furthermore, CYP51 protein modeling, according to the observed Cyp51 nucleotide substitutions, was performed and corresponding conclusions were used in the interpretation of this correlative analysis.
Resistance to azole (DMI), SDHI and strobilurine class fungicides in field isolates of *Zymoseptoria tritici* in Baltic countries

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In Estonia, outlined by the ECRI, the specific monitoring program (http://monitoring.etki.ee) for detection of leaf diseases on cereals is carried out since 2014 to undertake control measures when first symptoms detected to minimize the impact of disease. In Estonia the 2-spray strategy using 0.75 dose of fungicide is used to control *Zymoseptoria* in wheat.

In 2018 in addition to monitoring we also started fungicide resistance-related investigations. A total number of 63 isolates from Baltic countries including 29 from Estonia, 4 from Latvia and 30 samples from Lithuania were tested for sensitivity to epoxiconazole, prothioconazole-destio, tebuconazole and fluxapyroxade. This was the first year of testing and therefore there is no reference to previous years. In addition 10 samples from Finland were included into the study. Sensitivity testing was carried out as bioassay analysing single spore isolates on different concentrations of fungicides.

The average EC50 value in Baltic countries (and Finland) to prothioconazole-desthio varied between 0.06–0.24 ppm, tebuconazole 1.25–17.7 ppm, and epoxiconazole 1.04–1.98 ppm. The samples were also analysed for CYP51 mutations. Frequencies of D134G, V136A/C, A379G, I381V, and S524T in the Finnish-Baltic region were lower than in other Nordic countries.

The average EC50 value for fluxapyroxade varied between 0.07–0.32 ppm. The isolates were analysed for SDHI mutations. SDHI mutations C-N86S, C-N86K, C-T79N, C-T79I, C-W80S, C-G90R, C-H152R were not found in the Finnish-Baltic region in 2018.

The frequency of mutation G143A conferring strobilurin resistance was high (50-70%) in all of samples from Estonia, Finland, Latvia and Lithuania.

Those findings demonstrate that although the presence of fungicide resistance mutations has been confirmed for azole and strobilurin class fungicide these mutations do not dominate (except G143)A in the Baltic region and there has been no reports in the failure of the field performance of these fungicides so far.

This abstract was presented as a poster at Location 19
Impact of pre-crop and pre-pre-crop on the development of winter wheat leaf diseases

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Winter wheat leaf diseases cause serious yield losses globally. Agronomic practice is considered an important tool to reduce the development of these diseases. The aim of the present study is to evaluate the impact of a pre-crop and a pre-pre-crop on the severity of wheat leaf diseases under different soil tillage methods. Observations were made in a field experiment established at the Study and Research farm “Peterlauki” of the Latvia University of Life Sciences and Technologies in 2008. The data obtained from 2012 to 2018 (except 2014) were analysed. The development of winter wheat leaf diseases was assessed in a two-factorial experiment: 1 – soil tillage (ploughing; disc harrowing); 2 – different pre-crops and pre-pre-crops (wheat, wheat; wheat, oilseed rape; oilseed rape, wheat; barley, faba beans; wheat, faba beans). Severity of diseases was determined 5–7 times during vegetation, from BBCH 33 till 77, and the AUDPC (area under diseases progress curve) was calculated. Tan spot (Pyrenophora tritici-repentis) and Septoria leaf blotch (Zymoseptoria tritici) were the most widespread diseases during the whole investigation period; however, the severity of diseases differed depending on meteorological conditions. The levels of mildew (Blumeria graminis) and yellow rust (Puccinia striiformis) were too low to evaluate the influence of agronomic practices. Soil ploughing slightly decreased tan spot development but did not influence the level of Septoria leaf blotch. Continuous wheat essentially increased the level of tan spot, but differences between other variants of crop rotation were not significant. Crop rotation scheme had no effect on the development of Septoria leaf blotch. Crop rotation and soil tillage methods influenced the level of winter wheat leaf diseases, but a complex approach is required to control these diseases.

Acknowledgments: The research was supported by the grant from the Ministry of Agriculture of the Republic of Latvia “Influence of minimal soil tillage on its fertility maintenance, development and distribution of pests as well as crops’ yield and quality in resowings”.

This abstract was presented as a poster at Location 20.
Tan spot, caused by *Pyrenophora tritici-repentis* (Ptr), is one of the most destructive foliar wheat diseases worldwide. It has become more frequent in Tunisia over the last decade. Infected wheat leaves were collected from durum and bread wheat fields in the northern part of the country where cereal production is concentrated. In this study, the virulence of 74 isolates of Ptr was evaluated on four differential wheat genotypes. Polymerase chain reaction tests with *ToxA* and *ToxB*-specific primers were conducted to confirm the presence or absence of these genes in tested pathogen populations. In total, 37 (50%) isolates amplified both *ToxA* and *ToxB* genes, and those isolates induced typical symptoms of race 7 where both necrosis and chlorosis developed on corresponding susceptible wheat genotypes. However, the majority of the 35 (47%) isolates which amplified *ToxB* only, induced necrosis on ‘Glenlea’, and therefore these isolates do not fit the established Ptr race system. Ptr necrosis is typically induced by Ptr *ToxA*, but here the involvement of other necrosis inducing factor(s) is evident. In conclusion, Ptr pathogenic populations in Tunisia are mainly Ptr *ToxB*-producers and they do not necessarily follow the established race system, and additional unidentified necrosis factor(s) are most likely involved in pathogenicity.

This abstract was presented as a poster at Location 21
In the current global context of chemical use reduction in agriculture, new alternative crop protection methods have to be developed. One of the most promising solutions is the use of biocontrol products for the management of plant diseases. The goal of our work is therefore to identify new biocontrol compounds efficient on the wheat-Zymoseptoria tritici pathosystem and to characterize their modes of action. A high-throughput screening of a panel of more than 200 biomolecules or extracts of diverse origins and natures (bacterial and fungal extracts, glycolipids, lipopeptides, rhamnolipids, etc.) has been performed. Their direct antifungal activity has been evaluated using in vitro fungal culture and their indirect activity has been assessed on wheat plantlets by targeting chosen biomarkers involved in plant defence reactions against biotic stress. Preliminary results revealed that some biomolecules and extracts exhibit significant direct activity (total or partial fungal inhibition) that varies depending on the nature and the origin of each compound. Current investigations are in progress to determine minimal inhibitory concentration (MIC) and half-maximal inhibitory concentration (IC50) for each active compound. Furthermore, analyses of enzymatic assays focusing on key enzymes of defence pathways are performed to determine the potential defence activation by the compounds. The most active biomolecules or extracts (by taking into account both direct and indirect activities) will be selected for further analyses in order to characterize more precisely their modes of action.

This abstract was presented as a poster at Location 22
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Zymoseptoria tritici (previously Mycosphaerella graminicola) is the causal agent of Septoria tritici leaf blotch (STB), an economically important disease that significantly reduces wheat (Triticum aestivum) yields. During infection, the fungus enters leaves via natural openings, the stomata, and completes its full asexual reproductive cycle without penetrating host cells. Depending on nutrient availability, and temperature, Z. tritici can grow in vitro in either a "yeast-like" budding form or a hyphal form as well as switch between these forms. The ability of this pleomorphic fungus to grow in vitro in a yeast-like form is unusual for filamentous fungi and enables functional genomics studies aimed at the isolation of morphogenic switching genes, many of which contribute to fungal virulence. Furthermore, Z. tritici is amenable to several mutagenesis techniques, such as Agrobacterium-mediated transformation and UV-mutagenesis. The ability to rapidly generate strain libraries as well as the compact nature of its fully sequenced haploid genome (~37Mb) aids mutation discovery. This project will characterise existing mutant libraries, and generate new libraries, with the aim to identify novel virulence and avirulence genes via a mutagenesis-coupled genome resequencing approach. The best candidate genes affecting virulence or avirulence will be subjected to validation analysis (through independent targeted gene deletion and phenotyping) and then studied in further detail to elucidate their mechanistic roles.
The objectives of this study are to isolate *Zymoseptoria tritici* races, causal agent of Septoria tritici Blotch (STB), from infected wheat leaves and using DNA extracted from these races to construct a pangenome of *Z. tritici* in the UK. The material used in this study are derived from STB-infected wheat leaves from four locations across the UK. Infected leaves from 30-40 cultivars from each environment were collected in 2018 and will be collected again in 2019 and 2020. 15 isolates of *Z. tritici* are collected from each environment each year. These isolates make an environment specific inoculum which are then sprayed on blocks of five winter wheat cultivars at growth stage 39. Scoring occurs at 28 days post inoculation for percentage leaf area diseased. The inocula are sprayed on blocks individually or in mixes of two environments. The five cultivars are Andante, Exsept, Flame, Longbow, and Stigg.

The 2018 inoculation yielded the following scores from each inoculum or mixture: Swindon 14.6±8.8%; Bishop Burton 9.3±6.4%; Carnoustie 8.8±5.9%; Lenham 11.1±9%; Swindon and Bishop 12.2±22%; Carnoustie and Lenham 7.7±2.3%.

The *Z. tritici* isolates will be genotyped by next generation sequencing. The genomic information from the pathogen will be used to build a pangenome across the three years, allowing the examination of the overall UK *Z. tritici* pangenome, and how it is changing across space and time.

We would like to acknowledge Origin Enterprises-SFI for funding this research.

This abstract was presented as a poster at Location 24.
**Structure analysis uncovers a novel effector family related to *Ustilago* killer proteins in *Zymoseptoria tritici***

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*Zymoseptoria tritici* has a large number of secreted protein effectors coding genes, most of which are expressed during infection of wheat leaves. We determined the 3-dimensional structure of *Z. tritici* effector Mycgr3-91409. Previous structure-based HMM pattern searches suggested that Mycgr3-91409 is the only representative of the MAX (*Magnaporthe* AVRs and *ToxB*) effector family in *Z. tritici*. MAX effectors have a conserved β-sandwich fold and highly diverse primary protein sequences. Mycgr3-91409 was expressed in *E. coli* without its signal peptide and the purified protein was analysed for its 3D structure by NMR spectroscopy, and X-ray crystallography. Mycgr3-91409 had an α/β-sandwich structure completely different from MAX effector, but similar to KP6 killer proteins from *Ustilago maydis*. KP6α and KP6β are forming a protein complex toxic to non-producing fungi. Accordingly, Mycgr3-91409 was named Zt-KP6-1. Zt-KP6 orthologs were identified in *Z tritici* populations and in closely related species *Z. brevis*, but not in other fungi. Survey of Zt-KP6-1 genetic diversity, indicated that it was subjected to purifying selection, suggesting a functional role for this protein. A paralog of Zt-KP6-1, Mycgr3-96389, was identified in *Z. tritici* and in related species *Z. pseudotritici* and *Z. ardabiliæ*, but not in other fungi. Structure modeling suggested that Mycgr3-96389 had the same protein fold as KP6α and Zt-KP6-1, and it was named Zt-KP6-2. RNAseq data indicated that Zt-KP6-1 and Zt-KP6-2 were not expressed during fungal growth *in vitro*, but they were highly expressed during the asymptomatic phase of wheat leaf infection. Therefore, these two proteins are interesting candidate effectors, expressed specifically during infection. It will be interesting to determine whether these effectors have a toxic activity on fungi and/or plants. These experiments also clearly showed that determining the 3D structure of effectors, is a powerful method to assign these proteins to families.

This abstract was presented as a poster at Location 25
Investigating the genetic diversity of the fungal wheat pathogens *Zymoseptoria tritici* and *Fusarium graminearum* from field site isolates

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The global demand for wheat is expected to increase by 33% by 2050 (FAO, 2006). In Ireland, wheat is the second largest cereal produced and constitutes 30% of the 2.4 million tonnes of cereal produced (CSO, 2017). Two major pathogens which threaten wheat production are the fungi *Zymoseptoria tritici*, causal agent of Septoria Tritici Blotch (STB) and *Fusarium graminearum* causal agent of Fusarium Head Blight (FHB).

This project aims to use data collected from *Z. tritici* and *F. graminearum* field populations to investigate genetic diversity and identify effector gene candidates which may be required for disease. Fungal spore traps have been installed at four sites across the UK (Angus, Yorkshire, Wiltshire and Kent) and samples have been collected bi-weekly from May-August in 2018 and this will continue until 2022. The spore trap samples from 2018 are currently being microscopically examined for the presence of *Z. tritici*, *F. graminearum*, and other disease-causing fungi. There are more than 13 fungal species on the 2018 samples. Among the most abundant fungal spore types identified include: *Alternaria* spp., *Cladosporium* spp., *Fusarium* spp., *Didymella* spp., *Puccinia* spp. and *Z. tritici*. Between the sites there is a difference in the abundance of these fungal species. DNA has been extracted from the samples and sent for sequencing to assess *Z. tritici* and *F. graminearum* strain diversity.

The data collected from this project will be used alongside fungal field isolate studies in order to identify key virulence genes in *Z. tritici* and *F. graminearum* required for disease. The results from this research will be used to inform future control strategies against these major pathogens of wheat.

This abstract was presented as a poster at Location 26
During their life cycles, pathogens have to adapt to many biotic and abiotic environmental stresses to maximize their overall fitness. Morphological transitions are one of the least understood of the many strategies employed by fungal plant pathogens to grant survival. We characterized the responses of *Zymoseptoria tritici* to a series of environmental stresses to understand the effects of changing environments on fungal morphology and adaptation. This led to the discovery that *Z. tritici* forms chlamydospores. Though chlamydospores were especially prominent in one of the tested strains (1A5), we observed chlamydospore production in all four strains (1A5, 1E4, 3D1, and 3D7) both *in vitro* and *in planta*. We demonstrated that chlamydospores are better able to survive extreme cold, heat, drought and are also the most resistant morphotype to fungicides. Moreover, we demonstrated that the chlamydospore formation ability is present worldwide. We also used the natural morphological variation among *Z. tritici* strains to determine the genetic architecture of cell morphology by quantitative trait loci (QTL) mapping. We phenotyped 230 offspring isolates from the cross between 1E4 (mainly hyphal growth) and 1A5 (only chlamydospore formation) at 27°C. QTL mapping analysis identified a 95% confidence interval containing only eight genes. Two of them encoded transcription factors (TF1 and TF2) and another two encoded phosphatases that were highly polymorphic among the parental strains. Functional characterization of both transcription factors indicated that they are putative repressor of hyphal growth. We hypothesize that the alleles from 1A5 lead to a complete repression of the morphological transition to hyphal growth in 1A5, which results in chlamydospore formation as an extreme stress response morphotype. Our results illustrate that a foliar wheat pathogen produces chlamydospores that enable the pathogen to survive under highly stressful conditions. Natural genetic variation enabled us to identify new genes involved in the morphological stress response.

This abstract was presented as a poster at Location 27
Host-Pathogen Interactions

ABS21211

Comparison of genetic diversity of *Zymoseptoria tritici* populations on Tunisian durum wheat landraces and the modern variety Karim

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Septoria leaf blotch (STB) caused by *Zymoseptoria tritici* is one of the most damaging fungal pathogen on durum wheat. The genetic diversity of *Z. tritici* populations has already been the subject of numerous studies but no study has compared the genetic diversity of these fungal populations present on genetically diverse hosts such as landraces and on a genetically homogeneous host such as a modern variety. In this context, campaigns of collection of *Z. tritici* isolates were performed targeting the landraces Chili and Mahmoudi and the most cultivated modern Tunisian variety Karim in three localities from North Tunisia, “Joumine”, “Lansarine” and “El Jouf”, where small-scale farmers still grow such varieties next to each other. Four hundred and two *Z. tritici* isolates were collected between 2017 and 2018, and analyzed with 12 microsatellite markers. Most isolates were collected on Karim as the nearby landraces remained free from septoria infection; only one population of isolates could be collected from a single field on Mahmoudi at “Joumine”. To understand this strong contrast, the landraces and Karim were sown in a field trial in the northern Tunisian locality “Mateur” where STB pressure is usually very high; STB severity was high on Karim while the landraces remained, once more, almost immune. Further work will consist in characterizing the genetic diversity existing between the different *Z. tritici* populations to assess the effect of genetic host diversity on the diversity of pathogen populations in interaction with the location. This will lead us to better understand why Tunisian durum wheat landraces are so efficient in limiting STB epidemics.

This abstract was presented as a poster at Location 28
The fungal pathogen *Zymoseptoria tritici* is the causative agent of *Septoria tritici* Blotch (STB) which is one of the most devastating diseases of wheat (*Triticum aestivum L*), particularly in Northern Western Europe.

The effectiveness of commercial fungicides as a control mechanism against STB is decreasing, therefore there is a need to identify novel methods to control STB. X-ray Computed Tomography (CT) is a non-destructive imaging technique, which enables *Z. tritici* to be visualised in 3-dimensions *in planta* during infection.

Our aim is to combine molecular techniques with X-ray CT scanning to investigate *Z. tritici* infection in the host and in the non-host grass *Brachypodium distachyon*. Preliminary data shows pycnidia can be visualised using X-ray CT imaging. From this, fundamental new knowledge will be gained on the life cycle and structure of *Z. tritici* and enable phenotyping of the disease. Understanding a non-host interaction will inform future resistance strategies to *Z. tritici*. This phenotyping method of visualising fungal structures can be used for comparing the disease progression in wheat and in non-host grass systems.

This abstract was presented as a poster at Location 29
Investigating the role of the early wheat immune system to Zymoseptoria tritici fungi using bio-imaging approaches

Wheat (Triticum aestivum), one of the most widely grown crops, plays a pivotal role in the daily intake of nutrients. Despite the great progress in the agrochemical industry and the high fungicide input in the past 50 years, phytopathogenic fungi still represent the main biotic threat for crop security.

The hemibiotrophic fungus Zymoseptoria tritici (Z. tritici), the main causal agent of the foliar disease Septoria tritici blotch (STB), is characterised by a symptomless biotrophic phase lasting 7-11 days, followed by a necrotrophic period with development of fungal asexual sporulation structures called pycnidia throughout the leaf surface. Devoid of the presence of intracellular feed structures, Z. tritici is able to penetrate the host following hyphae germination exclusively via stomatal sub-cavities within 12 hours post infection.

However, several aspects of the first latent phase, including the active role of the early wheat immune system, and the spatiotemporal detection of the hyphae at the subcellular scale at the stomatal complex, remain still uncertain. Herein, we are currently testing the hypothesis that the early wheat immune system responds to pathogenic detection via early changes in cell wall architecture and organelle re-arrangements at the stomatal complex. In order to address our biological question, we propose a model of host-microbe interaction using bio-imaging approaches based on live-cell imaging and fluorescent proteins labelling organelles to investigate early sub-cellular responses occurring in wheat during the early interaction with Z. tritici strains.

This study could elucidate the potential role that the early wheat immune system might deploy at the stomatal apparatus in hyphae perception through cell wall reinforcement and organelle clustering at the site of fungal penetration.
Resistance in winter wheat to Septoria leaf blotch, caused by *Zymoseptoria tritici* (*Zt*), can be governed by the gene-for-gene interaction. Thus new races of *Zt* could appear and render some forms of cultivar resistance ineffective, as is the case for the cereal rusts and mildews. In practice under field conditions, it is rare to see a change in race that causes a variety to become more susceptible. In 2015 in the UK, the variety Cougar, rated as the most resistant cultivar in use at the time, became markedly more susceptible to Septoria leaf blotch in different locations across the country. To investigate this further, sentinel plots were established in the following season at breeding sites across the UK. Samples of Septoria leaf blotch were taken and from each sample, three isolates of *Zymoseptoria tritici* were collected. These were tested on seedlings of the cultivars Cougar and Gallant, the latter being a susceptible control, to classify isolates as being "Cougar"-type or "non-Cougar"-type. Five isolates of each were then tested further to assess their risk to other resistant varieties in the UK at both the seedling and adult plant stages. Alongside these tests, fungicide resistance assays were conducted and genotyping of isolates with a set of 21 microsatellite markers was performed. Genotyping data from the 2016 isolates demonstrated high levels of genetic diversity as expected for a sexually-reproducing organism, and it was not possible distinguish between Cougar and non-Cougar types based on genotypes alone. These experiments were repeated in 2017 and results will be presented to show the potential impact of such changes in pathogenicity along with hypotheses on the evolution of the Cougar- infecting isolates.

1 Gautier *et al.*, Development of a rapid multiplex SSR genotyping method to study populations of the fungal plant pathogen *Zymoseptoria tritici*. BMC Research notes 7:373
Over the past decades, plant breeders have focused much effort on the identification and deployment of durable resistance in crops. The rust resistance gene *Lr34* is an example of such a gene as it provides broad-spectrum resistance against leaf rust, stripe rust and powdery mildew in wheat\(^1\)\(^-\)\(^2\) and has been effective under field conditions for several decades\(^3\). However, there is now evidence that *Lr34* also has pleiotropic effects on the plant’s susceptibility to diseases such as Septoria tritici blotch and wheat blast in seedlings\(^4\). Such a trade-off also occurs with *mlo* mildew-resistance genes, which increase susceptibility to Ramularia leaf spot in barley\(^5\).

To reduce the damage caused by facultative pathogens to varieties with *Lr34*, it is necessary to assess the effect of this gene on a wide range of damaging diseases of wheat. If these trade-offs are apparent under field conditions, this will have major implications for breeding strategies.

We are testing the effect of *Lr34* on increasing susceptibility to wheat blast in glasshouse experiments. Our data show that both seedlings and adult plants of wheat carrying *Lr34* are more susceptible to wheat blast infection than plants without *Lr34*. Ears of *Lr34* lines infected by wheat blast produce grains with a significantly lower weight than non-*Lr34* lines. We are also studying the effect of *Lr34* on host responses to blast by transcriptome analysis of infected and uninfected wheat plants with or without *Lr34*.

References


This abstract was presented as a poster at Location 32
Pyrenophora tritici-repentis (PTR) induces tan spot, one of the most important fungal diseases of wheat. At least eight races of the pathogen are known to occur based on their virulence on the six standard wheat differential set. The ability to recognize the distinct symptoms (necrosis/chlorosis) produced by isolates of PTR on wheat had significant implications for genetic and wheat breeding programs since they helped to explain the basis for susceptibility to the pathogen. While races 1, 2, 3, 4 and 5 have all been reported from North America, more than 90% of the PTR isolates from this continent have been classified as races 1 or 2. Races 1, 2, 4, 5, 6, 7 and 8 have been reported from North Africa, while races 1, 2, 3, 5, 7 and 8 were found in a survey of the Caucasus and the Fertile Crescent regions. Limited surveys of central Asia revealed the presence of races 1 and 2 in that region which were the only ones detected in the Southern Cone Region of South America. The different races identified so far in each of those locations are a reflection of the wheat cultivars used locally and pathogen migration. The ability to easily and objectively characterize races on a qualitative system based on standardized protocols should be of direct benefit when screening for resistance and, eventually, in the development of tan spot-resistant cultivars, thereby allowing the design of effective resistance diversification strategies.
Characterisation of small RNA mediated silencing in the *Zymoseptoria tritici* - wheat interaction

Cross-kingdom small RNA mediated silencing (also known as RNA interference or RNAi) has recently been shown to occur in several plant-pathogen interactions. However, it remains unknown how universal this phenomenon is, and in particular whether RNAi facilitates colonization of plants by fungal pathogens that invade extracellular spaces and do not form specialized feeding structures or penetrate host cells either during entire life cycle or during prolonged initial phases of infection. One such pathogen is *Zymoseptoria tritici*, which causes Septoria tritici blotch - a serious foliar disease of wheat. In this study we defined the global small RNA (sRNA) populations in *Z. tritici* expressed *in vitro* as well at the several key time points during wheat colonisation, and computationally predicted their potential wheat mRNA targets. However, molecular approaches failed to validate targeting of selected wheat mRNAs by fungal sRNAs, and bioassays using newly generated *Z. tritici* mutant strains carrying DCL and AGO gene deletions suggested that these important components of RNAi are dispensable for full infection of wheat. Moreover, we demonstrated that *Z. tritici* is incapable of environmental dsRNA uptake, and neither *in vitro* nor *in planta* application of dsRNAs in a virus-mediated Host-induced gene silencing procedure was effective in preventing fungal growth or disease. Collectively, our data suggest that RNAi approaches for gene function analyses in *Z. tritici* and potentially also as a disease control measure may not be as effective as has been demonstrated for some other plant pathogenic fungi.

This abstract was presented as a poster at Location 34
Septoria tritici blotch (STB) caused by the fungus *Zymoseptoria tritici* is an important foliar disease of wheat in the UK. Symptoms of chlorotic and necrotic lesions with pycnidia appear on leaves 7-14 days after infection when the disease reduces plant photosynthesis. Plants have evolved photoprotective mechanisms to avoid photo-oxidative damage and dissipate excess energy that cannot be utilised by the photosynthetic machinery. Non-Photochemical Quenching (NPQ) is the process of heat dissipation to protect the integrity of photosystem (PS) II and it typically modulates plant responses to environmental stress. Dynamic changes of NPQ reflect tight regulation of energy required to drive photochemistry and to prevent permanent damage to PSII caused by reactive oxygen and free radicals. A recently cloned STB-resistance gene, *Stb6*, was found to encode a conserved wall-associated receptor kinase (WAK)-like protein that confers pathogen resistance without a hypersensitive response (HR). We hypothesized that upon infection with *Z. tritici*, wheat genotypes containing *Stb6* will exhibit NPQ dynamics associated with reduced oxidative stress in the absence of HR. Time series of chlorophyll fluorescence imaging of healthy and artificially inoculated wheat leaves as well as visual rating of disease severity were performed on wheat germplasm that either contained or lacked *Stb6*. NPQ induction and relaxation curves as well as a range of related physiological parameters were measured and correlated with disease severity. Significant down-regulation of NPQ in the absence of symptoms by the pathogen was observed in the resistant genotypes, suggesting that chloroplastic defence plays an important role in disease resistance. Further work will investigate the relationship between NPQ and oxidative stress in STB resistance.

This abstract was presented as a poster at Location 35.
Estimating the effect of leaf spot disease complex on grain yield, test weight and thousand kernel weight in a durum wheat population

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Leaf spot disease complex (LSDC) (tan spot, septoria leaf blotch, and spot blotch) is one of the most prevalent and widespread diseases affecting durum wheat production in Western Canada. Leaf spot diseases reduce the photosynthetic area of leaves resulting in reduced grain filling and lower yields particularly when the penultimate and flag leaves are infected. This study was conducted to estimate the losses caused by LSDC on grain yield, test weight, and thousand kernel weight in an adapted durum wheat population. Over four years, in a field near Swift Current, SK, we grew 167 doubled haploid durum wheat lines derived from a cross of commercial cultivars Strongfield and Pelissier. We evaluated them for natural LSDC infection using the 0-11 McFadden scale for the vertical progression of disease in the canopy. At maturity, plots were harvested and grain yield was measured. Kernel weight was determined on a sample of 1000 kernels randomly taken from the harvested grain. Test weight was determined gravimetrically on clean grain samples. Correlations and regression between LSDC reaction and grain yield, thousand kernel weight, and test weight were calculated. A significant negative correlation was observed between LSDC reaction and grain yield ($r = -0.4$, $P < 0.0001$, $n = 167$). Regression analysis showed that the average yield reduction was 108 kg ha$^{-1}$ for every increment of the disease in the canopy on the 0-11 scale. On average, yield losses reached 33.7% when the flag leaf is infected. Regression analysis on the lines with disease reaction higher than seven showed yield losses of 36.9%. LSDC showed a negative relationship with test weight and thousand kernel weight, but was not significant. The results demonstrate that greater susceptibility to LSDC is related to increased grain yield losses in durum grown in southwestern Saskatchewan.

This abstract was presented as a poster at Location 36.
The.Ptr-barley interaction is specific and is controlled by a single locus

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Tan spot caused by the necrotrophic fungus Pyrenophora tritici-repentis (Ptr) is a major foliar disease of wheat in North America. Previously, we showed that this pathogen can interact specifically with barley, and that the necrotrophic effector Ptr ToxB induces chlorosis in a highly selective manner when infiltrated into certain barley genotypes. In this study, a comprehensive genetic map composed of 381 SNP markers were used to map the locus conditioning this specific interaction in a population of 94 doubled haploid lines from a cross between Haruna Nijo and H602. Reaction to the race 5 isolate of the fungus, a Ptr ToxB-producer, was evaluated at the seedling stage in a greenhouse. The lines segregated 1:1 for susceptible: resistance phenotypes, indicating the involvement of a single locus. The locus was mapped to the distal region of the short arm of chromosome 2H in barley.

This abstract was presented as a poster at Location 37
Genetic determinism of quantitative pathogenicity in *Zymoseptoria tritici*

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*Zymoseptoria tritici* is a hemibiotrophic fungus responsible for the septoria leaf blotch (STB) disease on wheats and triticale. Strains of the fungus are not only host species specific, but also show specificity to varieties. This specificity is often explained by a gene-for-gene interaction between resistance genes in hosts and pathogenicity genes in fungi. This interaction is illustrated in the case of *STB6-AVRSTB6*, with *STB6*, a major resistance gene, and *AVRSTB6*, the corresponding avirulence gene in *Z. tritici*. Contrary to other pathosystems, the gene-for-gene interaction is not characterised by a hypersensitivity reaction. 21 major resistance genes and 89 resistance QTL have been mapped in wheat to date. Host resistance is predominantly polygenic and quantitative; however, gene-for-gene interactions are not well documented in the case of quantitative resistance. We therefore investigated quantitative pathogenicity in *Z. tritici*. The two *Z. tritici* strains INRA09-FS0732 and INRA09-FS0813 were isolated at Grignon in 2009. These strains were chosen for their differential pathogenicity on Renan, a bread wheat variety with a high level of resistance to STB. We crossed the two strains and obtained a population of 168 progeny entirely genotyped following a RAD-seq approach. The identification of SNP polymorphisms in the RAD-seq sequences by comparison with the genome sequence of the reference strain IPO-323, allowed for the construction of a dense genetic linkage map. The 168 progeny were inoculated on Renan and the phenotypes used to detect QTL for pathogenicity. These phenotypes were related to symptom development and fungal sporulation. Four QTL have been detected so far. The strongest and most reliable QTL was detected on *Z. tritici* chromosome 6 in a A/T and transposon-rich region. We will investigate how the gene underlying this QTL affects pathogenicity and whether it is implicated in a gene-for-gene interaction with a resistance QTL from Renan.
Breeding novel genes for resistance to *Zymoseptoria tritici* causing Septoria tritici blotch (STB) in wheat is essential for the future sustainable management of the disease. Phenotyping tools measuring chlorophyll fluorescence (CF), in addition to traditional disease assessments, can be deployed to screen plant populations for the early detection and characterisation of novel resistance. The aim of this study was to characterise and differentiate the effects of a novel resistance gene (RG) identified in the cv. Cougar by RAGT from the known resistance gene, *Stb6*. Sixteen genotypes from a population, cross between the winter wheat cv. Cougar and cv. Scout, containing all possible combinations of the novel RG and *Stb6* were spray-inoculated at GS 27 with *Z. tritici* (IPO323) and grown under controlled environment. The effect of the RG and *Stb6* on the efficiency of photosystem (PS) II was quantified by measuring CF parameters with two different instruments, MultispeQ (PhotosynQ) and Fluorpen (PSI). Pycnidia production, necrosis latency and disease progression were visually assessed from 7 days post inoculation. During the asymptomatic phase of the disease, at 4 hours post inoculation, RG lines exhibited reduced excitation energy flux trapped per active reaction centres and increased non-photochemical quenching (NPQ) compared to *Stb6* lines. Once symptoms were visible, NPQ in RG lines decreased significantly whilst quantum yield of PS II increased. RG lines were also characterised by reduced disease symptoms progression (AUDPC) and delayed development of pycnidia and necrosis. The results show that the resistance gene is highly effective against STB as early as GS27 (early tillering) and differ from *Stb6*. CF can be used to characterise the effects of the RG on the early photochemical reactions of photosynthesis consistent with reduced symptom expression. Future work will use NILs to investigate the physiological and molecular mechanism of this novel resistance gene.

This abstract was presented as a poster at Location 39
Zymoseptoria tritici, the causal agent of septoria tritici blotch (STB) disease of wheat (Triticum aestivum L.), continuously threatens Ireland and Europe’s wheat crop causing loses up to 50% if left untreated. STB is mostly controlled by applying fungicides causing an economic loss of > €1bn annually to EU. Consequently, strains of Z. tritici have evolved fungicide resistance, which is impeding effective control. In addition, EU legislation has removed several fungicides due to environmental concerns, which is further driving resistance to the remaining chemistries within Z. tritici populations. Alternatively, genetic resistance is the most sustainable strategy; however, most commercial wheat varieties lack adequate STB resistance. Therefore, new breeding methodologies are needed to rapidly identify new sources of resistance to STB. Here we apply genomic selection (GS) and speed breeding (SB) technology to a 16-founder wheat multiparent advanced generation inter-cross (MAGIC) population (termed ‘NIAB Diverse MAGIC’), comprising of > 600 F7 inbred lines, to accelerate the identification of new sources of STB resistance. The MAGIC population has been genotyped with a 35K SNP array, with ~1.2 million SNPs identified via founder exome capture and skim sequencing and imputation in the progeny currently in progress (NIAB, UCL). A subset of the NIAB Diverse MAGIC population encompassing maximum genetic diversity will be selected as a training population (~200 lines). The training population will be evaluated for STB resistance under SB to develop the prediction model using various algorithms (e.g. GBLUP, random forest, support vector regression and various Bayesian approaches), thus allowing us to capture the variance of the large effect quantitative trait loci (QTL) together with the variance for the genome-wide smaller effect QTL. The accuracy of predictions will be determined by correlating the genomic estimated breeding values (GEBVs) with the corrected phenotypes. GEBVs are then used to select candidates from the test population for advancement in the breeding cycle. The selected candidates will be subject to validation via SB and in the field at two sites in 2020 under high STB inoculum pressure. The accuracy of the models will be determined by correlating prediction accuracy via SB and in the field. The genomic regions identified via genome-wide association studies will be aligned with previously reported QTL and catalogued resistance genes on the new bread wheat genome reference sequence (IWGSC RefSeq v1.0), allowing rapid identification of candidate genes conferring STB resistance. We envisage identifying new sources of resistance to STB disease that thereafter will be integrated into European wheat breeding programmes.
Characterization of seedling infection types and adult plant infection responses of Tunisian durum wheat to prevalent races of *Pyrenophora tritici-repentis*

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Tan spot, caused by the necrotrophic fungal pathogen *Pyrenophora tritici-repentis* (Ptr) has become an increasingly important foliar disease of wheat worldwide. In Tunisia, this disease has the potential to drastically reduce yield and could become a major constraint to wheat production, particularly where continuous wheat is grown coupled with cultivation of susceptible cultivars under minimum or zero tillage. In Tunisia, there are six identified *P. tritici-repentis* races. The race structure studies showed wide virulence spectrum of tan spot disease in Tunisia. In this study, the two prevalent races 5 and 7 were used to characterize resistance levels at seedling growth stages of 150 Tunisian durum wheat accessions that included landraces and commercial cultivars. Same accessions were evaluated under field condition and natural infections at Koudia BouSalem research station (higher semi-arid). Different levels of resistance were observed amongst the collections tested. Detailed results will be presented. Developing durum wheat cultivars that are multi-race resistant to Ptr thus presents a significant challenge to breeders.

**Key words**: Tan spot, *Pyrenophora tritici-repentis*, resistance, durum wheat landraces, commercial cultivars.

This abstract was presented as a poster at Location 41
Septoria tritici blotch (STB) caused by Zymoseptoria tritici has become a major disease for durum wheat worldwide but particularly in the Mediterranean area. For a long time, control of STB was ensured by a wide range of fungicides and not much was done on host resistance until 1990’s due to the appearance of Septoria resistance to fungicides that is a clear risk to wheat production. Faced with this situation, durum wheat breeding programs launched intensive work in search for resistance. Up to date Septoria resistance genes were characterized on bread wheat but none or very few were characterized on durum wheat. CIMMYT with partners established a regional septoria phenotyping platform with major focus on screening durum wheat and aiming to provide information to collaborators. CRP Wheat led by CIMMYT supported the establishment, in partnership with the Government of Tunisia, of Septoria phenotyping platform that would lead the search for resistance to Z. tritici in close cooperation of stakeholders within Tunisia and NARS of North Africa as well as advanced research Institutions and Universities in Europe, USA, and Australia. Currently the platform has the potential to screen up to 20000 accessions annually at two distinct hot spot locations, complemented with artificial inoculation. Following first year round (year of testing) susceptible accessions are eliminated and the resistant ones are re-tested for second year information. In 2018-2019 season. Over nine thousand durum accessions from Tunisia (571) Algeria (223), CIMMYT (3394), ICARDA (1047), Bari-Italy (757), INRA France (195), Canada (287) and USDA (1570) are screened under field and growth chamber conditions. Good resistances were identified that would allow collaborators to further assess and eventually characterize resistance novel genes in durum wheat background. Capacity development and germplasm will be a major contribution of the platform at the local, regional and global level.

Keywords: Zymoseptoria tritici, durum wheat, resistance, CIMMYT, Phenotyping Platform
Characterisation of major genes mediating resistance to Septoria tritici blotch disease in wheat.

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The fungus *Zymoseptoria tritici* is one of the most destructive wheat (*Triticum aestivum*) pathogens in Europe and worldwide, causing crop losses of up to 50% in high risk climates (Goodwin, 2007). Traditionally this disease has been controlled with widely used resistance genes and fungicides, but the high selection pressures placed on the fungi result in a serious risk of these protections being overcome, particularly when heavily relied upon. Some major resistances have already been widely broken – for example, the Stb6 resistance gene present in most European wheat cultivars is now ineffective against many Septoria strains in the field.

It is therefore important that new, more diverse sources of resistance be identified and utilised in elite wheat lines. These will allow us to prepare for the breaking of currently common resistances but may also extend their lifetimes – Chartrain et al. (2004) found that many wheat lines with highly durable Septoria resistance contain multiple resistance (Stb) genes, suggesting that gene pyramiding may be a viable method for enhancing the longevity of resistances in this pathosystem.

The research described here will screen currently known Stb genes against an array of recent Septoria field isolates to identify resistances still effective in the field and potentially interesting combinations of resistances that in combination could provide protection against most or all isolates tested. Such resistances will then be fine mapped using KASP markers to enable breeders to more easily integrate them into elite lines. In light of the recent identification of the Stb6 gene as a wall-associated receptor-like kinase (WAK) (Saintenac et al., 2018), WAK genes in the regions identified will be further investigated using Virus-induced gene silencing to identify individual resistance genes where possible, aiding in further investigations that may establish the methods through which these resistances function.

This abstract was presented as a poster at Location 43
Identification and characterisation of novel wheat genes that confer resistant to Septoria Tritici Blotch disease during latent phase of the infection

Septoria tritici blotch (STB) disease affecting wheat is caused by the fungal pathogen Zymoseptoria tritici. Early detection and initiation of defence responses during the latent phase of infection are crucial in allowing the host to prevent successful pathogen infection. RNAseq data from a doubled-haploid population derived from a cross between cvs. Stigg and Longbow representing the 96-hour response to the pathogen was conducted and a suite of early-response genes have been identified for further characterisation. A second bulk segregant analysis (BSA) RNAseq, composed of two bulks representing the 10-day response to the pathogen was conducted. The first bulk was comprised of six elite resistant cultivars and the second bulk comprised of six elite susceptible cultivars. Following the analysis of the BSA RNAseq data, a variety of candidate genes were identified to be involved in defence against STB. Induction of genes associated with catalytic activity and metal binding were prevalent in the resistant bulk compared to susceptible bulk. Expression of a set of homoeologous Reticulata-like genes were downregulated in the resistant bulk only suggesting, based on studies in other species, a potential burst of reactive oxygen species (ROS). Thirty-five common genes were identified across the two RNAseq datasets, including an adenosylhomocysteinase, a glutathione S-transferase and a serine-type endopeptidase inhibitor. This study shows that defence responses are conserved among wheat cultivars and highlights the importance of the early host response.

This abstract was presented as a poster at Location 44
Fine mapping of Stb19, a new resistance gene to Zymoseptoria tritici in wheat

A new R gene, Stb19, was mapped on the short arm of chromosome 1D, exhibiting complete resistance to three Zymoseptoria tritici isolates, WAI332, WAI251, and WAI161 at the wheat seedling stage. Two closely linked Kompetitive Allele Specific PCR markers with Stb19, snp_4909967 and snp_1218021, were then tested and validated in an additional segregating population and a collection of 266 wheat accessions, giving 95% accuracy of resistance/susceptibility prediction. Stb19 was also integrated into four commercial wheat cultivars to increase the performance of resistance in the field. Towards cloning the gene, Stb19 was fine mapped in a mega base pair region based on IWGSC reference genome.
Recruiting resistance to Septoria tritici blotch disease from a wild relative of wheat

Septoria tritici blotch (STB) of wheat is a devastating foliar disease caused by the fungal pathogen *Zymoseptoria tritici*, accounting for significant yield losses in temperate regions worldwide. This pathogen has overcome all unisite fungicides and evolved multidrug resistance; therefore, it is vital that new resistance genes are discovered, characterised and deployed. Many crop wild relatives contain unexploited genetic variation which has not been subjected to the genetic bottlenecks and strong selection pressures imposed upon modern monoculture-grown crop varieties. The D-genome progenitor of bread wheat, *Aegilops tauschii*, represents a rich source of disease resistance that can be readily incorporated into wheat. Many *Ae. tauschii* accessions are highly resistant or immune to STB; however, understanding the genetic basis of this resistance is hindered by a poor agronomy which complicates the creation of laboratory-based genetic population structures. To overcome this challenge, we are using the historical recombination accumulated in a genetically diverse, sequence-configured population of *Ae. tauschii* ([www.openwildwheat.org](http://www.openwildwheat.org)). We will present our work so far on using genome-wide association genetics to characterise the functional genetic architecture of *Ae. tauschii* resistance to *Z. tritici*.

This abstract was presented as a poster at Location 46
Dissection of resistance components against Septoria tritici blotch in a common wheat line 'Murga'

Septoria tritici blotch (STB) is a major foliar disease globally, which is notorious in the fast development of fungicide resistance, making host resistance an indispensable component in mitigating STB. CIMMYT wheat line ‘Murga’ is well known for its high, durable and broad-spectrum resistance against STB infection, and the purposes of this study were to investigate its resistance mechanism and identify molecular markers that could be used in marker-assisted selection (MAS). A recombinant inbred line population was derived from a cross between ‘Murga’ and a STB susceptible line ‘Huirivis’, comprising 297 progenies. The population was evaluated for STB resistance in Toluca, Mexico (2017 and 2018), and in INIA Estanzuela, Uruguay (2016 and 2017). STB disease severity (STBds) was visually scored in field trials artificially inoculated with STB isolates. Plant height (PH) and days to heading (DH) were scored in all the experiments. Genotyping was performed using the DArTSeq technology. QTL mapping (the MQM algorithm in MapQTL) indicated a major and stable QTL on chromosome 3DL, explaining a phenotypic variation for STBds of 41-63% in Mexico and 28-30% in Uruguay. This QTL is close to Stb16q, as indicated by the physical positions of its flanking markers in the Chinese Spring reference genome. Eight location-specific QTL with minor effects were identified: two on 2D and 5B only expressed in Mexico, and six on 2B, 2D (two QTL), 3A, 3B and 5B only expressed in Uruguay. ‘Murga’ was the resistant donor for all QTL, except for those on 2B and 3A. When PH and DH were used as covariates, the major QTL on 3DL was not affected, whereas the minor QTL were affected to various degrees. Flanking markers of the 3DL QTL will be transformed into KASPs to evaluate their suitability for MAS.

This abstract was presented as a poster at Location 47
Contribution of proteinaceous effectors in *Parastagonospora nodorum* blotch development in wheat and triticale.

*Parastagonospora nodorum* is a necrotrophic pathogen of wheat and triticale. It is known to produce several protein effectors which cause necrosis development in susceptible host genotypes. Pathogen can incite necrosis of leaves as well as glumes. Destruction of green plant parts affects photosynthesis adversely, what results in grain yield loss, quantitative and qualitative in nature.

Among Polish *P. nodorum* population, Tox1, Tox3 and Tox5 are most frequent effectors. ToxA is present only in minority of screened isolates. All of these effectors were purified and used to phenotype wheat and triticale lines. Diversified breeding materials of wheat and triticale were utilized in disease evaluation trials in field and controlled environment conditions. Tox3 and Tox5 insensitivity were positively correlated with phenotypic resistance. Elimination of Tox3 sensitive lines as well as lines sensitive to other effectors can subsequently be used to increase *P. nodorum* resistance in wheat and triticale breeding materials.

This abstract was presented as a poster at Location 48.
The fungus *Parastagonospora nodorum* is a necrotrophic pathogen of triticale in many parts of the world. It causes disease by secreting proteinaceous effectors which interact with proteins encoded by dominant susceptibility genes in the host. The outcome of these interaction results in the pathogen affected plant tissue allow the fungus to thrive and survive on dead plant material. During last years appeared reports on proteinaceous host selective toxins produced by *P. nodorum* in infected plant tissue. The Tox3 is the most common toxin produced by isolates collected in Poland. In this study, an effort was undertaken to compare seedlings of winter triticale somaclonal and dihaploid lines and conventional varieties according to *P. nodorum* resistance and sensitivity to Tox3 among.

Nine triticale cultivars, nineteen somaclonal lines and twenty-two double haploids produced from cultivars varying in resistance to *P. nodorum* were evaluated under controlled environment conditions. The first seedling leaves were inoculated with a mixture of *P. nodorum* isolates. After 10 days of incubation, the disease severity on seedling leaves was rated on a disease severity scale, where >90% – susceptible, <10% – resistant. The differences between seedling leaves for all components were statistically significant. Higher resistance to *P. nodorum* was observed more often on leaves of somaclonal lines then on dihaploid ones. Some of genotypes were showing low leaf infection, e.g. dihaploid D-46 and somaclone S-43. A correlation between the triticale line sensitivity to Tox3 toxin with phenotypic susceptibility to *P. nodorum* was observed at the seedling stage. In conclusion, we have demonstrated that somaclonal variation might be used as an additional source of triticale germplasm enhancement according to its resistance to the pathogen in question. The use of Tox3 test to identify and eliminate susceptible triticale genotypes has clear advantages and it can be recommended to use in breeding programs.
Identification of resistance sources and Genome wide association study of Septoria tritici blotch at seedling and adult plant stages in spring bread wheat germplasm of ICARDA

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Septoria tritici blotch (STB) of wheat, caused by the ascomycete Zymoseptoria tritici (formerly Mycosphaerella graminicola) is one of the most important foliar disease of wheat. In Morocco, STB is a devastating disease in temperate wheat growing regions and the yield losses can exceed up to 50% under favorable conditions. The aims of this study were to identify sources of resistance in a set of 377 advance breeding lines (ABLs) of spring bread wheat breeding program of ICARDA, and to identify loci conferring resistance to STB both at seedling as well as at adult plant stage. Seedling resistance was evaluated under controlled conditions with two virulent isolates of STB (SAT-2 and 71-R3) from Morocco. Where as, adult plant resistance was assessed at two hot spot locations in Morocco (Sidi Allal Tazi, Marchouch) under artificial inoculation with a mixture of STB isolates. At seedling stage, 45 and 32 ABLs were found resistant to 71-R3 and SAT-2 isolates of STB, respectively. Interestingly, 13 ABLs were found resistant to both STB isolates. At adult plant stage, 50 ABLs were found resistant at both hot spot locations in Morocco. Furthermore, 6 advance breeding lines (2, 68, 89, 133, 164 and 276) showed resistance at seedling as well as at adult plant stage. The genome wide association studies (GWAS) revealed a total of 33 QTL at seedling stage (25 for SAT-2 and 8 for 71-R3 isolate), where as, 7 QTL were detected at adult plant stage. Identification of STB resistant spring bread wheat germplasm in combination with QTL found at both stages, will serve as an important resource in STB resistance breeding efforts.

This abstract was presented as a poster at Location 50
Spot blotch (caused by Bipolaris sorokiana) and terminal heat stress are the two major issues for substantial yield reduction and economic losses of wheat in the warm and humid regions of the world particularly EGP of South-Asia where wheat is grown in around 10 million hectares. An investigation was conducted with an aim to determine the combined effect of terminal heat stress and spot blotch of wheat. Fifty five diverse wheat genotypes were grown in two dates of sowings (timely and late sown) under two treatments - inoculated with B. sorokiana and protected with azoxystrobin during 2016-17 and 2017-18. The experiment was aimed to observe the combined effect of terminal heat stress and spot blotch on wheat in relation to membrane damage and effect on grain characters and various quantitative traits such as plant height, spike length, thousand kernel weight, biomass and grain yield as well as several and physiological traits (chlorophyll content, waxiness index, NDVI, MSI and MDA). The study revealed oxidative damage due to combined stress of spot blotch and terminal heat stress that caused increase in MDA content leading to greater damage to membrane that was further responsible for increased electrolytic leakage. MSI was lower in susceptible genotypes. AUDPC had significant positive correlation with lower values of AU(SPAD)DC and AU(NDVI)DC under both timely and late sown conditions. The decrease in chlorophyll content was 20% due to combined effect of spot blotch and heat stress. Reductions in various grain traits due to combined or individual effect of spot blotch and heat stress was obtained. For instance, the combined effect of two stresses caused 63% reduction in biomass whereas spot blotch alone caused 38% reduction. Study revealed that spot blotch along with terminal heat is causing significant yield losses and therefore both need due attention by wheat breeders.
Investigating the role of the Circadian Clock in the wheat fungal pathogen, *Zymoseptoria tritici*

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The fungus *Zymoseptoria tritici* causes Septoria Tritici Blotch (STB), one of the most devastating diseases of wheat in Europe which accounts for up to 40% yield loss. There are currently no fully durable methods of control against *Z. tritici*, so novel strategies are urgently required.

The purpose of this research is to investigate whether the circadian clock is important for pathogenicity and development in *Z. tritici*. Circadian clocks are molecular machineries which are entrained by environmental signals such as light and temperature. Previous research has shown that the host circadian clock can regulate defence against pathogen attack. However, research from the pathogen perspective is limited.

In order to identify the core circadian clock components in *Z. tritici*, known fungal clock genes were BLAST searched against the genome database of this pathogen. The expression patterns of the potential *Z. tritici* clock components identified are currently being investigated using a combination of RNAseq and RT-qPCR studies. These candidate genes are being knocked-out in *Z. tritici* via Agrobacterium-mediated transformation, and the phenotypes assessed in vitro and *in planta*. To date, two of the mutant lines tested have displayed developmental defects with possible implications in pathogenicity.

This study is one of the first documented investigations into the role of the circadian clock in an economically important cereal pathogen. Findings from this research will impact future control strategies against *Z. tritici* such as timing of fungicide application, disease severity measurements, and genes for future fungicide target screens.

This abstract was presented as a poster at Location 52