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Investigating sources of resistance in wheat to septoria tritici blotch disease

Key external stakeholders:

Tillage farmers, enterprises focussed on wheat breeding

Practical implications for stakeholders:

Septoria tritici blotch (STB) disease poses the single greatest disease threat to Irish winter wheat acreage. Caused by the leaf pathogen *Zymoseptoria tritici,* STB poses a consistent challenge to European wheat production, with approximately 70% (> €400 million) of annual cereal fungicide input focussed on mitigating STB-associated losses. The issue is compounded for farmers by the emergence and prevalence of fungicide-resistant/insensitive strains of *Z. tritici* and the lack of any durable STB resistance in existing varieties. Output from this study has begun to address this issue by (i) field screening pre-breeding and commercial material to identify sources of STB resistance and (ii) employing biotechnology-based strategies to investigate how wheat responds at the early stages of STB infection.

The outcome/technology or information/recommendation is that:

- Varietal material does exist with strong resistance to STB disease
- A suite of genes whose response is unique to the resistant phenotype has been identified.
- Armed with this knowledge it will be possible to generate novel lines of wheat with STB resistance

Main results:

- The field performance of multiple varieties and breeding lines of winter wheat was assessed for STB resistance across three separate seasons.
- Varietal material does exist with the ability to provide consistent levels of high resistance to STB.
- Employing gene expression analysis, the transcriptomic response of a resistant cultivar (Stigg) versus that of a susceptible cultivar (Gallant) in response to *Z. tritici* has identified a unique suite of genes, which represent targets for future efforts to develop STB resistant varieties

Opportunity / Benefit:

Output from this project has generated strong datasets to support the future development of winter wheat varieties with durable resistance to STB disease. This is of most relevance to farmers and stakeholders in all sections of the cereal trade.

Collaborating Institutions:

UCD

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External collaborators:	Prof. Fiona Doohan, UCD

1. Project background:

STB disease is the single most important disease threat to wheat achieving its yield potential in Ireland. A deficit of durable resistance in existing varieties has seen the Irish wheat sector depend solely on fungicide application to preserve yields, at an average national cost in excess of €14 million. While average responses to fungicidal control are ~ 4 t/ha, more recent fungicide strategies have been significantly eroded through the emergence of *Z. tritici* strains with resistance to the strobilurins and triazole (e.g. tebuconazole, epiconazole and prothioconazole) based chemistries.

Across Europe, the situation is further complicated by the reversal of protection-product registrations from a risk-based to a hazard-based assessment, as per Regulation (EC) No 1107/2009, which is likely to see the prohibition of the most effective STB-focused fungicides. Consequently, reliance on the remaining succinate dehydrogenase inhibitor (SDHI) fungicides can be expected to increase. However, their sustainability is already questionable due to their site-specific mode of; indeed, in 2015 strains with SDHI resistance were identified by Teagasc in Irish wheat fields. In short, the future of winter wheat production in Ireland is becoming increasingly unsustainable, which highlights the necessity to adopt a multi-disciplinary approach to identify novel disease control strategies for STB disease in Ireland.

A hemibiotroph, *Z. tritici* infects solely via host stomatal cavities, with penetration occurring within 12 h post-inoculation. Growing inter-cellularly, infecting hyphae advance into the mesophyll layer during an asymptomatic phase with no evident haustoria or other specialised feeding structures recorded to date. At 12–20 dpi, this latent period ends and *Z. tritici* switches to an aggressive necrotrophic lifestyle that leads to the characteristic STB lesions which bear spore-producing pycnidia along the leaf surface. This manifestation of symptoms in wheat is a result of a system of programmed cell death (PCD) in the wheat leaf which involves a loss of cell integrity. For the wheat-*Z. tritici* interaction this PCD process facilitates pathogen growth and sporulation by providing a source of nutrients to *Z. tritici*. The asexual life cycle continues with the transfer of pycnidiospores through the crop canopy via rain splash, explaining why STB thrives so well in temperate and humid climates.

The impact of STB disease on yield formation in wheat is primarily through reduced persistence of the crop's green canopy (primarily the last 3 leaves) during the grain filling period. At a genetic level, thirteen major genes (Stb1 to Stb12 and Stb15) for resistance to STB have been identified, with the Stb6 and Stb15 genes most prevalent in European varieties at present. Yet, the field performance of these genetic resources has been eroded due to the ability of *Z. tritici* to evolve and generate novel more virulent strains.

In response, this project had two research objectives: (i) evaluate pre-breeding and commercial germplasm in addition to ecotypes of the model cereal *Brachypodium distachyon* to identify material with STB resistance and (ii) assess the genetic response of identified resistant material after exposure to *Z. tritici* with the goal of identifying single and/or multiple gene(s) and/or gene networks associated with STB resistance.

2. Questions addressed by the project:

- Can wheat material with the potential for adaptation to the extreme disease pressures prevalent in the Irish agri-environment be identified?
- Can transcriptomic analyses provide novel insight into how wheat responds to *Z. tritici* infection during the symptomless stages of infection?
- Is it possible to employ *Brachypodium distachyon* as a model species to study the initial stages of infection?

3. The experimental studies:

Field studies were completed in Oak Park between 2010 and 2013 with up to 34 varieties/breeding lines assessed for cultivar performance against STB and resulting yields in the presence and absence of a commercially derived 3 spray fungicide regime. Each treatment x variety interaction was replicated 4 times across a replicated randomized design, with 10 leaves per plot randomly selected for assessment at three time points during the growing season; thereby providing disease scores at 3rd, 2nd and flag leaf stages of plant growth. To investigate wheat's genetic response to *Z. tritici* infection, a comparative analysis of the transcriptome response of cv. Stigg versus that of cv. Gallant was completed using the Affymetrix 61k Wheat GeneChip Array. This platform allows for the identification of genes differentially expressed between the partially resistant Stigg genome and the highly susceptible Gallant wheat genome. For this experiment, the gene expression differences between the two cultivars were examined across five time points: 0, 1, 4, 8, 12 and 14 days post infection. To determine the suitability of the model grass species *B. distachyon* for studying the wheat-STB host pathogen interaction, five inbred lines (Bd1-1, Bd12-3, Bd21-1, Bd29-1 and Bd30-1) were treated with a pycnidial suspension of IPO323 (~5ml) using a hand-held spray bottle. Treated plants were covered with polythene bags for 7 days to ensure high humidity and symptom progression was recorded at 1, 4 and 7 dpi and classified as (i) no symptoms, (ii) patches of yellow and red chlorosis, (iii) chlorosis with necrotic lesions or spots or (iv) necrosis with no symptoms of chlorosis. The wheat cv Riband, was included as a positive control to ensure *Z. tritici* spore viability and isolate virulence.

4. Main results:

Trials conducted in 2010-2011 experienced moderate levels of disease pressure and lower mean yields in comparison to the 2011-2012 trial. In 2010-2011, Avatar had the lowest untreated disease score and the lowest difference in yield. Varieties Stigg, Warrior and Alchemy also performed well; with moderately low disease scores coupled with moderately low yield losses. Though vars. Einstein, Consort and Riband had high levels of STB, yield losses were low. Varieties Claire and JB Diego performed poorly; with moderately low and high untreated disease scores respectively but also suffering yield losses of greater than 2.5 t/ha. The trial conducted in 2011-2012 had the highest levels of disease and also the greatest difference in mean yields between fungicide-treated and untreated plots. During this season, Stigg had both the lowest untreated disease score as well as the lowest response to fungicide (Figure 1). Warrior, Riband, Alchemy and JB Diego recorded moderately high levels of disease with yield losses less than 2.5 t/ha (Figure 1). The highest fungicide responsive varieties in 2011-2012 were Oakley and Avatar (Figure 1).

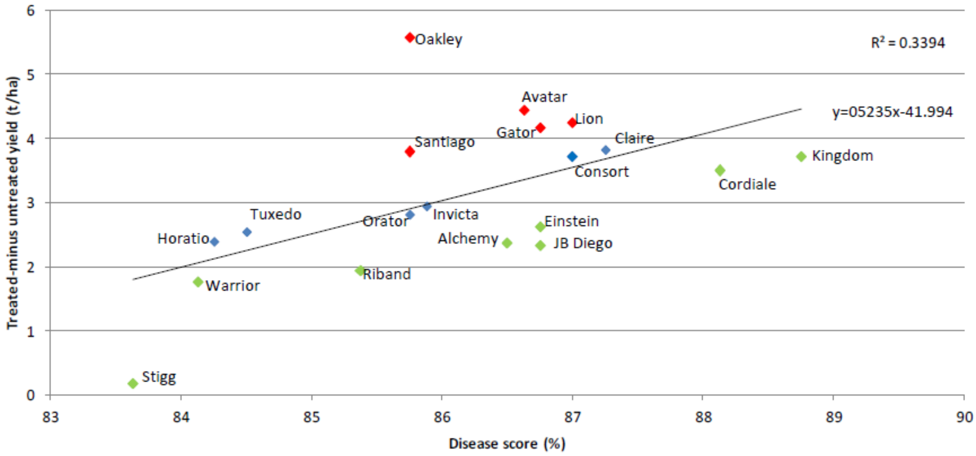

Figure 1. Untreated disease scores (%) for leaf 4 versus differences in yield (treated-untreated yields) for the 2011-212 season. Low (green), moderate (blue) and high (red) untreated disease scores for 19 varieties

The 2012-2013 study experienced low disease pressure and moderate yields due to warm temperatures. Invicta had the lowest untreated disease score with a yield reduction followed by Gator, Lion, Warrior and Avatar, with yield losses of less than 0.5 t/ha. Einstein, Kingdom and Orator and Horatio performed well, with moderately high to high levels of disease and differences in mean yield of less than 1 t/ha. Of interest the poorest performing varieties in this season were Stigg and JB Diego; both had moderate levels of disease but suffered the highest yield losses of 1.7 t/ha and 2.1 t/ha respectively.

Studying the genetic response to STB, a bimodal pattern of gene induction was noted for Gallant with gene activity peaking at 1 dpi and 12 dpi, which coincides with the initial period of infection and the biotrophic-necrotrophic switch by the pathogen. In contrast, gene expression in Stigg peaked at ~8dpi. At 4dpi, strong patterns of cell wall fortification took place in cv. Stigg with this being the primary biological process in this cultivar at this timepoint. At 12 dpi the most striking observation in cv. Stigg was the activity of cell wall related genes, with 7 genes expressed > 50 fold. It is known that *Z. tritici* can possibly direct wheat gene expression to increase the porosity of the plant cell wall thereby increasing nutrient availability to *Z. tritici*. However, this study has shown that Stigg responds by up-regulating the activity of wall fortifying proteins to reinforce the cell wall and prevent nutrient loss to the pathogen.

Investigating the *B. distachyon* – *Z. tritici* interaction, it was observed that for Bd21-1 symptoms were visible from 1 dpi and these developed into blotch-like lesions by 4 dpi. These lesions then became necrotic with chlorotic regions expanding up to 7 dpi (Figure 2). However, sporulation on *B. distachyon* tissues was not observed and no evidence of fungal penetration could be obtained. This contrasted with the wheat control var. Riband, where symptoms developed from 12 dpi with pycnidia abundant by 20 dpi. Significantly, observations of host responses to *Z. tritici* IPO323 in five *B. distachyon* ecotypes revealed a variation in resistance responses, ranging from immunity to a chlorotic/necrotic phenotype.

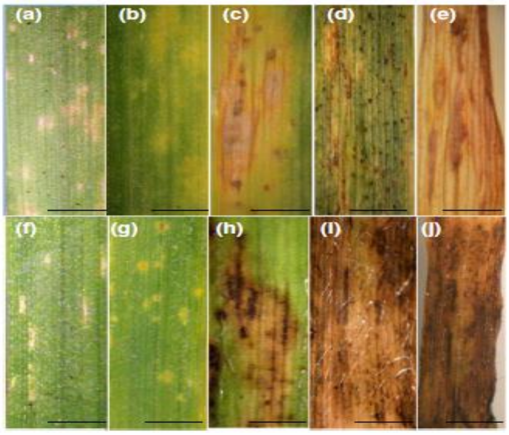

Figure 2 Septoria tritici blotch symptoms on the wheat cultivar Riband and the *Brachypodium distachyon* ecotype Bd21-1. Plants were treated with a pycnidical spore suspension of the *Zymoseptoria tritici* isolate IPO323 (spore concentration: 1x10-6/ml) at the 2 leaf stage (cv. Riband) and the 6 leaf stage (Bd21-1). (a-e). Symptoms were recorded on cv. Riband at 12, 14, 17, 20 and 31 days post inoculation respectively. (f-j) Symptoms were recorded on Bd21-1 at 1, 4, 7, 14 and 21 days post inoculation respectively. Scale bars = 0.5 cm.

5. Opportunity/Benefit:

The work completed in this project highlighted the range of genotypic resistance which is present in current germplasm while also identifying the limitations with respect to their durability of resistance in varying environments. This should provide an impetus for breeding companies to focus on integrating those selected varieties that possess strong genetic resistance to STB disease into their breeding programmes. The present study has also revealed that genes involved in cell wall durability behave very differently in resistant and susceptible material, with results indicating that cell wall fortification might indirectly/directly determine a resistant phenotype against *Z. tritici*. As a result, several gene targets have been identified for follow on investigations. Finally, it was noted that STB symptom development on *B. distachyon* is comparable to that observed during the early infection stages on wheat. However first visible symptoms occurred more rapidly on *B. distachyon*; from 1 dpi in comparison to 12 dpi in wheat. Consequently, the interaction between *B. distachyon* and *Z. tritici* as observed in this study could serve as a suitable model pathosystem with which to investigate mechanisms underpinning a resistant response to *Z. tritici*.

6. Dissemination:

Scientific output generated during the project was disseminated at national and international scientific meetings

Main publications:

O'Driscoll, A., Kildea, S., Doohan, F., Spink, J. and Mullins, E. (2014). The wheat–Septoria conflict: a new front opening up? Trends in Plant Science, Vol.19, Issue 9, September, pp 602–610.

O'Driscoll, A., Doohan, F. and Mullins E. (2015). Exploring the utility of *Brachypodium distachyon* as a model pathosystem for the wheat pathogen - *Zymoseptoria tritici*, BMC Research Notes, 8, pp132

O'Driscoll A. An investigation into the genetic mechanisms underpinning host resistance to Septoria tritici blotch disease in wheat. PhD Thesis, UCD.

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