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Monitoring Pathogen Evolution for Sustainable Cropping

Key external stakeholders:

Tillage farmers, Tillage Advisors (Teagasc & Independent), Agrichemical industry, Regulators, Researchers

Practical implications for stakeholders:

The major pathogens of Irish wheat, barley and potato crops all exhibit the potential to develop fungicide resistance and virulence to host resistance. Continued monitoring is therefore essential to ensure the continued effectiveness of fungicides and varietal resistances. In addition:

- Large collections of *Phytophthora infestans*, *Zymoseptoria tritici* and *Rhynchosporium commune* have been established and are available for future studies on fungicide sensitivity or host virulence.
- The future deployment of varietal late blight resistance may lead to changes in the late blight population, including leading to more aggressive strains of late blight.
- Resistance to the SDHIs in the Irish *Zymoseptoria tritici* population and to the QoI fungicides in the Irish *Rhynchosporium commune* population has been confirmed and as such careful consideration of fungicide programme design is required to minimise the spread and impact of these resistances.
- Irish *Z. tritici* populations exhibit high levels of genotypic diversity, indicative of sexual reproduction and as such the development and spread of fungicide resistance and/or virulence can be expected.

Main results:

The Irish *P. infestans* population continues to be dominated by strains belonging to the clonal lineages EU_13_A2, EU_6_A1 and EU_8_A1. Selection for strains of the more aggressive lineages EU_13_A2 and EU_6_A1 occur on the more resistance potato varieties (e.g. Sarpo Mira, Bionica), whilst the current fungicides used do not support selection within the current *P. infestans* population. Overall, evidence indicates that genetically, the Irish populations of *P. infestans* are relatively simple with a few clonal lineages. A high throughput sequencing assay was developed to screen *R. commune* populations for the mutation G143A, known to confer QoI fungicide resistance. The mutation was detected in 2013 and 2014, albeit at low frequencies. The assay designed can be used on various platforms ensuring its transferability. Resistance to the azole fungicides in the Irish *Z. tritici* population was investigated and found to be conferred by target site alterations/overexpression and enhanced efflux activity. Significant differences in sensitivity to the different azoles exist in the population, which can be manipulated with fungicide programmes. Resistance to the QoI fungicides continues to dominate the *Z. tritici* population, whilst resistance to the SDHI fungicides was first detected in 2015. Population analysis using SSR markers also confirmed high levels of gene flow within the Irish *Z. tritici* population, indicating high levels of sexual reproduction in the population. No evidence for host adaptation was identified in the Irish population, although evidence of adaptation due to geographic location was detected.

Opportunity / Benefit:

For each pathogen extensive collections were established and are an asset for future studies. The molecular assays developed will continue to be used in both monitoring studies and field trials. In addition the genomes of 90 *Z. tritici* isolates were sequenced and are available for future investigations.

Collaborating Institutions:

University College Dublin (UCD); Agri-Food and BioScience Institute (AFBI)

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1. Project background:

Globally, Ireland consistently achieves some of the highest tillage yields due to a moist maritime climate during the growing season. Yet, volatile commodity prices, increased regulatory constraints and the emergence of highly aggressive, fungicide-resistant strains of the primary crop pathogens threaten the sector's competitiveness. Wheat, barley and potato are the three primary tillage crops on the island of Ireland but all three succumb to significant disease pressures that growers counter with the use of high fungicide inputs. Unsustainable in the long-term, the current strategies have led to an accelerated rate of genetic change in pathogen populations. As a consequence, novel strains of *Phytophthora infestans* (potato late blight), *Zymoseptoria tritici* (septoria tritici blotch of wheat) and *Rhynchosporium commune* (barley leaf scald) have emerged, which present a significant challenge to the continued viability of each cropping system. In response, this project aims to counter this issue by completing (i) a robust pathogen monitoring strategy for each disease and (ii) a complementary programme that disseminates in real time to stakeholders viable alternative strategies to mitigate the impact of changing pathogens.

2. Questions addressed by the project:

- What is the current fungicide sensitivity and population status of the major pathogens of wheat (*Zymoseptoria tritici*), barley (*Rhynchosporium commune*) and potato (*Phytophthora infestans*)?
- To what extend has the Irish *Phytopthora infestans* population the potential to develop virulence to newly deployed varietal resistances
- Can high throughput sequencing technologies be used to detect mutations conferring fungicide resistance or virulence in bulk samples?
- How does the Irish *Zymoseptoria tritici* population adapt to the cultivation of winter wheat varieties differing in resistance?

3. The experimental studies:

The project was split into two specific work packages.

WP1: Monitoring the Irish *P. infestans* populations for changes in fungicide and host resistances

Extensive collections of *P. infestans* were established both as live cultures and as DNA collections on Whatmann FTA cards. These included collections from commercial crops both in the Republic of Ireland and Northern Ireland, from the Teagasc breeding programme field trials and from late blight fungicide trials established as part of the project. DNA was extracted from all samples and their genotype determined using a standardized SSR assay. The sensitivity of a representative collection of isolates belonging to the dominant genotypes was determined to the CAA fungicide mandipropamid, the QiI fungicide cyazofamid and the phenylamide metalaxyl using a combination of molecular and in vitro assays. For the CAA a set of plasmids incoperating the different potential mutations known to potentially confer resistance were created for use as standards in future molecular assays.

WP2: Monitoring Irish *Z. tritici* and *R. commune* populations for changes in fungicide sensitivity and host resistance

Collections of both *Z. tritici* and *R. commune* were established in each season of the project (2013-2018). These included collections from commercial crops, both pre- and post-fungicide application, and field trials established to determine the impact of fungicide resistance on fungicide efficacy. Sensitivity was determined using a microtiter plate assay or molecularly using target site sequence analysis of fungicide specific target sites. Briefly *R. commune* lesions were excised, quantified and pooled together after which PCR was used to

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amplify a section of the R. commune cytochrome b encompassing codons 129, 137 and 143 and sequenced. *R. commune* populations from 74 crops from the 2006-2014 seasons were screened. To provide further insights on the development of azole resistance in the Irish *Z. tritici* population a detailed analysis of sensitivity to six azoles was conducted on a subsample of the 2015 collection. In addition, the CYP51 of each isolates was sequenced and the presence of inserts in both the promoter of both the CYP51 gene and MgMfs1 gene determined using PCR assays. To confirm the impact the presence of inserts have on the regulation of CYP51 expression, qPCR assays were conducted on a range of isolates in the presence and absence of the azole fungicide epoxiconazole.

To determine how the Irish *Z. tritici* population responds to the cultivation of varieties differing in host resistance a collection of isolates was established from four varieties varying in STB resistance grown in both Oak Park and Cork. Initial analysis was conducted using 24 SSR markers. Using a subset of the isolates, differences in aggressiveness between them was determined by re-inoculating them back onto each of the four varieties under controlled conditions. The genomes of 90 of these isolates were also sequenced for future reference. As three of the test varieties do not have the Stb6 resistance gene the corresponding effector AvrStb6 was sequenced in the entire isolate collection and compared to a globally publically available collection. To screen the wider Irish population for differences in AvrStb6 a high throughput amplicon sequencing assay was developed using Ion Torrent sequencing and applied to pooled DNA extracted from lesions excised representing 54 commercial crops during the seasons 2012-2017

4. Main results:

WP1: Monitoring the Irish *P. infestans* populations for changes in fungicide and host resistances

Over the course of the study a total of 2,416 single lesions were genotyped. Three clonal lineages, EU_13_A2, EU_6_A1 and EU_8_A1 dominated the population and were detected in each season. A small number of strains of the lineage EU_5_A1 were also detected. It was found that fungicide (10 different fungicides) did not notably affect the genetic structure of the population compared to the untreated control. In contrast, lesions sampled from more resistant potato varieties were more often found to be the more aggressive EU_13_A2 and EU_6_A1 compared to more susceptible potato genotypes. The resistant varieties Sarpo Mira and Bionica selected showed strong selection for EU_13_A2. Results of the present study indicate that, in natural *P. infestans* populations, EU_8_A1 EU_6_A1 have not been displaced by EU_13_A2 in the local population and remain competitive, but there is a continued need for effective control of *P. infestans* genotypes, such as EU_13_A2, with a high fitness on resistant potato lines.

WP2: Monitoring Irish *Z. tritici* and *R. commune* populations for changes in fungicide sensitivity and host resistance

Over the course of the study a continued erosion of sensitivity of the Irish *Z. tritici* to the azole fungicides was observed. This erosion was associated with target site (CYP51) mutations, target site overexpression and enhanced efflux activity. Strains combining the mutations V136A, I381V and S524T now dominate the population. Equally strains with one of three inserts in the CYP51 promoter region also dominate, however analysis of CYP51 expression confirmed only those with a 120 bp insert exhibited overexpression, and these we confined to a single CYP51 genotype. In late 2015 strains exhibiting resistance to the SDHI fungicides were first detect in the field and resulted from mutations (T79N or H152R) in their SDHI target site SDHC. Intensive sensitivity testing in 2016-2018 confirmed the spread of these mutations and others (notably N86S) through the Irish *Z. tritici* population. Field trials conducted in 2017 and 2018 confirmed a loss in efficacy of all currently available SDHI as a result of this. Using the intensive collections established in both 2016 and 2017 the status of QoI resistance in the Irish *Z. tritici* was determined. As expected resistance remains extremely high. Using the high throughput sequencing assay the presence of the cytochrome b mutation G143A was confirmed in the Irish R. commune for the first time in 2013 and subsequently in 2014.

SSR analysis of the *Z. tritici* collections established from the four winter wheat varieties grown in both Oak Park and Carlow confirm high levels of gene flow occur in the Irish *Z. tritici* population, indicative of a high level of sexual reproduction. Although no evidence for selection or adaptation was detected on the core chromosomes using the SSR assay, there appeared to be a significant effect of variety on the presence/ absence of the accessory chromosome 17. Although three of the host varieties did not have the STB resistance gene, all isolates were deemed virulent to the gene based on the AvrStb6 sequence analysis of each isolate. A sequencing assay was developed and used to characterize the AvrStb6 gene bulk samples of excised *Z. tritici* lesions from 54 commercial crops from 2012-2017. In the majority of crops a single virulent Avrstb6 haplotype dominated, although small differences in the frequency of other haplotypes were detected. Re-inoculation experiments of a selection of these isolates found no differences in aggressiveness between them depending on what variety they originated from. However a significant site effect was detected, with isolates from Carlow more aggressive compared to those from Cork.

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5. Opportunity/Benefit:

Baseline collections provide an invaluable resource to develop diagnostic assays to detect strains possessing effectors that can breakdown specific resistance in potato and fungicide resistance in cereals.

6. Dissemination: Selection of key events/publications

Across the 4 years of MonPESC project staff generated a total of 69 dissemination events/publications including:

Knowledge Transfer events:

- Septoria Conference 22nd March 2017: Preserving current and future control
- Septoria Crop Walks: Carlow (17th June 2014), Meath (19th June 2014), Cork (20th June 2014)
- Septoria Crop Walks: Meath (3rd July 2017), Cork (5th July 2017), Laois/Carlow (7th July 2017)
- Tillage Crops Open Day, Oak Park Carlow: 26th June 2013; 24th June 2015; 28th June 2017
- Fungicide sensitivity and disease control. *National Tillage Conference 2013 P53-60*
- Cereal disease control. *National Tillage Conference 2014 P91-102*
- Wheat disease control and resistance issues. *National Tillage Conference 2016 P39-46*
- Fungicide resistance in Irish cereal crops. *Agrichemical Representatives, Oak Park* (26th May)
- Fungicide resistance in Irish cereal crops. *PCRD, DAFM* (26th May)
- Fungicide resistance in Irish *Zymoseptoria tritici* update. *PCRD, DAFM* (5th December)
- Fungicide resistance in Irish *Zymoseptoria tritici*. *Syngenta Solatenol Launch* (9th-10th) December

Main publications:

Kildea S, Heick T, Grant J, Mehenni-Ciz J & Dooley H (2019) A combination of target-site alterations, overexpression and enhanced efflux activity contribute to reduced azole sensitivity present in the Irish *Zymoseptoria tritici* population. European Journal of Plant Pathology 154: 529-540

Kildea S, Bucar DE, Hutton F, de la Rosa S, Welch TE & Phelan S (2019) Prevalence of QoI resistance and mtDNA diversity in the Irish *Zymoseptoria tritici* population. Journal of Agriculture and Food Research 58: 27-33

Stellingwerf JS, Phelan S, Doohan FM, Griffin D, Bourke A, Hutten RCB, Cooke DEL, Kildea S & Mullins E (2018) Evidence for selection pressure from resistant potato genotypes but not from fungicide application within a clonal *Phythphthora infestans* population. *Plant Pathology* 67: 1528-1538

Welch T, Feechan A & Kildea S (2018) Effect of host resistance on genetic structure of core and accessory chromosomes in Irish *Zymoseptoria tritici* populations. European Journal of Plant Pathology 150: 139-148

Dooley H, Shaw MW, Mehenni-Ciz, Spink J and Kildea S (2016) Detection of *Zymoseptoria tritici* SDHI insensitive field isolates carrying SdhC-H152R and SdhD-R47W substitutions. *Pest Management Science 72:2203-2207*

Conference Papers:

Stellingwerf J, Doohan F & Mullins E (2014) Oomycete Molecular Genetics Network Meeting 2014

Phelan S & Kildea S (2016) Proceedings of the CPNB Conference 2016

Kildea S, Dooley H, Phelan S, Spink J & O'Sullivan E (2016) Proceedings of the CPNB Conference 2016

Kildea S, Dooley H, Phelan S, Mehenni-Ciz J & Spink J (2016) Modern Fungicides & Antifungal Compounds 16

Popular publications:

Kildea S (2013) Understanding the past to protect the future. *IFJ Crop Protection Supplement*

Kildea S (2014) What does reduced triazole sensitivity mean for field control. *IFJ Crop Protection Supplement*

Kildea S (2016) SDHI Resistance – the Irish experience. *IFJ Crop Protection Supplement*

Thesis:

Stellingwerf J (2017) Investigating the potential adaptation of *Phytophthora infestans* in Ireland against resistance in its primary host *Solanum tuberosum*. University College Dublin.

Welch T (2018) Monitoring changes in the Irish *Zymoseptoria tritici* population to host resistances. University College Dublin

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