An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

# Research Stimulus Fund

## Final Report

***'Monitoring Pathogen Evolution for Sustainable Cropping (MonPESC)'***

**DAFM Project Reference No:** 11/S/113

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**Principal Coordinator and Institution:** Dr. Steven Kildea, Teagasc
**Email:** stephen.kildea@teagasc.ie

**Collaborating Research Institutions and Researchers:**
*Teagasc - Dr. Steven Kildea, Dr. Ewen Mullin, Dr. Sinead Phelan (Post-Doctoral Researcher)*

*University College Dublin (UCD) - Prof. Fiona Doohan, Dr. Angela Feechan, Dr. Tomas McCabe, Thomas Welch (PhD student), Jeroen Stellingwerf (PhD student)*

*Agri Food and BioSciences Institute (AFBI) - Dr. Louise Cooke, Dr. Gillian Young, Dr. Lisa Black, Dr. Richard O'Hanlon*

**Please place *one* "x" below in the appropriate area on the research continuum where you feel this project fits**

| Basic/Fundamental → | | | Applied → | | | Pre Commercial |
|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 x | 6 | 7 |

**Please specify priority area(s) of research this project relates to from the National Prioritisation Research Exercise* (NRPE) report;**

| | |
|---|---|
| **Priority Area (s)** | I. Sustainable Food Production and Processing |

**Key words:** Pathogen virulence, fungicide resistance, *Phytophthora infestans*, *Zymoseptoria tritici*

## 1. Rationale for Undertaking the Research

In 2011 the national (Republic of Ireland) wheat, barley and potato crops presented respective farm gate values of €112 million, €197 million and €62 million per annum, yet the annual cost of protecting these yields (via disease mitigation) on wheat, barley and potato equates to €12 million, €14 million and €5 million respectively. The primary targets of these control strategies are *Zymoseptoria tritici* in wheat, *Rhynchosporium commune* in barley and *Phytophthora infestans* in potato. Without these intensive control measures the economic viability of each crop would be eliminated. However, this reliance upon fungicide inputs is vulnerable to changes in the sensitivity of target pathogens, a vulnerability which increases in tandem with the increasing specificity of modern fungicides. The emergence of resistance to existing fungicides and the ensuing compromised field performances threaten the sustainability of our current crop systems. Similarly, the development of stronger varietal resistance, while environmentally positive, will also exert strong selection pressure on each pathogen to evolve; thereby undermining the efficacy of the introduced resistance genes. At present, in an effort to preserve yields, cereal and potato growers make use of programmes of disease control that are both economically and environmentally unsustainable in the long-term. By acting in a knowledge vacuum, growers are not cognisant of the population dynamics of each of the three target pathogens: *Z. tritici*, *P. infestans* and *R. commune*. As such, current cropping regimes maybe accelerating the rate of genetic change of each, to the detriment of growers' returns. To address these issues, MonPESC establishes a comprehensive monitoring strategy for each pathogen, coupled with a detailed understanding of how resistant varieties are impacting upon the respective pathogen populations. MonPESC will deliver sound and robust control strategies to the sector and provide appropriate recommendations to preserve the integrity of existing (and future) varietal resistances and/or fungicide chemistries.

## 2. Research Approach

To aid the implementation of the different components of the project in an efficient manner MonPESC was divided into three specific sub-projects reflecting the different objectives of the project.

Sub-project 1: Monitoring the Irish *Phytophthora infestans* populations for changes i n fungicide and host resistances.

Sub-project 2: Monitoring Irish *Zymoseptoria tritici* and *Rhynchosporium commune* populations for changes in fungicide sensitivity and host resistance.

Sub-project 3: Development of strategies to counter selection of fungicide-resistant and host resistance-breaking strains of *Phytophthora infestans*, *Zymoseptoria tritici* and *Rhynchosporium commune*.

Although each sub-project and associated tasks dealt with different pathogens and/or different areas of research, where possibly continuity was sought between them.

**Subproject 1:**

Collections of *P. infestans* were established from commercial potato crops throughout Ireland and from intensive sampling of Teagasc potato fungicide and breeding trials conducted at Oak Park Carlow. The collections were established either as live cultures or through FTA cards which preserve the specific strains DNA. Using the standardised SSR assay each collection was genotyped and assigned to specific clonal lineages/genotypes. Using representative isolates from each their sensitivity to the CAA fungicide mandipropamid and the QiI cyazofamid was determined using an agar plate assay and/or *in vitro* sensitivity screens. For both fungicides analysis of the target gene sequence was determined, and in the case of the CAA a hybprobe assay was developed to detect potential mutations previously identified as potentially conferring resistance. Intensive sampling and genotyping of the late blight trial (described below sub-project 3) was also used to determine the potential impacts different fungicides may have on local *P. infestans* populations

Intensive sampling of the Teagasc potato breeding trials and plots made available to the project through the participation of Teagasc in the AMIGA project, and subsequent genotyping using the standardised SSR assay was conducted to determine the impacts of cultivar resistance on local *P. infestans* populations. Detailed *in vivo* experiments were conducted using Irish *P. infestans* isolates to determine their susceptibility to the Rpo-vnt1 resistance gene. To further dissect the capacity of the Irish *P. infestans* population to adapt to the introduction of novel resistance genes the sequencing technique Pathseq was applied to Irish *P. infestans* strains representative of the dominant clonal lineages present in Ireland. This method provides a detailed effector profile of the studies Irish strains identifying the diversity that may exist even within the small number of clonal lineages currently present in Ireland.

**Subproject 2:**

Extensive sampling of commercial wheat and barley fields and fungicide trials in 2013-2017 were conducted as part of the MonPESC project. From these *Z. tritici* and *R. commune* isolates, fungal DNA and pooled leaf/pathogen DNA collections were established. These have been maintained as part of the Teagasc Oak Park Phytopathological collection. Each season, sensitivity to the *Z. tritici* collection was conducted to fungicides representative of the different fungicide classes currently used. In addition intensive fungicide screens for wider range of fungicides belonging to both the azole and SDHI fungicides groups were conducted on collections established in 2015 and 2017. In collaboration with a Teagasc funded Walsh Fellowship student the sensitivity of *R. commune* collections were established to representative fungicides of the azoles and QoIs. For both pathogens sequence analysis of the genes encoding the fungicide target sites (CYP51, SDHB, C, D, β-tubulin and cytochrome *b*) were sequenced in isolates varying in sensitivity to the different fungicides. To aid the detection of mutations in these genes associated with fungicide resistance molecular detection methods were developed. These

included traditional and real-time PCR assays, KASP markers and high throughput sequencing assays based on 454- and ion-torrent sequencing.

To monitor how *Z. tritici* populations respond to the introduction of varietal resistance field trials with four winter wheat cultivars differing in their susceptibility (from extremely susceptible to extremely resistant) were intensively sampled in both Carlow and Cork, sites representing differing disease pressure environment. Any impacts of location and cultivar of origin on the diversity of the collection was determined using an SSR assay developed by Gautier et al. (2014). The presence/absence of the various accessory chromosomes was also screened using a PCR assay previously described by Croll et al. (2013). Given the limitations associated with SSRs in identifying potential evidence of population structure in a highly sexual fungus such as *Z. tritici* a sub-collection of the isolates (n=96) were sent for whole genome sequencing. To further identify if cultivars are impacting on local *Z. tritici* populations 111 isolates from the collection established were re-inoculated onto a panel of winter wheat cultivars (cvs Cordiale, Dunmore, Einstein and Stigg). Finally as the sequence of the effector Avrstb6 involved in conferring susceptibility/resistance towards wheat varieties with the resistance gene Stb6 became available during the life of the project the sequence of the gene in the *Z. tritici* isolates established from the four cultivars was determined. These sequences where compared to one another based on their cultivar of origin or location. Further analysis of the collection was determined using the sequences of collections from Australia, Israel, Switzerland and the USA that are publically available. To determine if changes in the diversity of Avrstb6 has changed in recent years the pooled field DNA collection established from commercial fields was screened using an amplicon sequencing assay.

Gautier et al. (2014) BMC Res Notes 7: 373
Croll et al. (2013) PLoS Genet. 9(6): e1103567

**Subproject 3:**

Reflective of the pathosystems investigated three trial were conducted as part of the MonPESC project. These trials were designed to reflect findings generated in subprojects 1 and 2 and to provide samples for each sub-project. In addition to the field trials, dissemination and outreach were included in subproject 3.

*Late blight trial:*

A field trial was conducted at Teagasc Oak Park, Carlow in 2014, 2015 and 2016. In each year the efficacy of 12 fungicide products applied at 7-10 day intervals and representing eight different fungicide groups was determined. The impacts of the fungicides on local *P. infestans* populations was determined by analysing the *P. infestans* genotypes present following treatment using an SSR assay (as described in Subproject 1).

*R. commune* trials:

Following the detection of G143A which confers QoI resistance in *R. commune* in both 2013 and 2014 a field trial was conducted at Knockbeg Research Farm in spring/summer 2014. In spring 2014 the mutation had been detected at the trial site. The impacts of nine

fungicide treatments, including the various QoIs available on selection of the allele were investigated using the 454-sequencing assay developed in Subproject 2. In addition in collaboration with the Teagasc funded Walsh Fellowship the impacts of mixing azole, QoI or SDHI fungicides on *R. commune* was investigated using field trials established in 2015 and 2016.

*Z. tritici* trials:
Following the detection of SDHI resistance in the Irish *Z. tritici* population in late 2015 and 2016 a series of winter wheat field trials were established to investigate the impacts of SDHI resistance on efficacy and selection. The trials were established in Carlow, Cork and Meath and the main SDHI fungicides were applied as solo treatments or in combination with prothioconazole or chlorothalonil. Intensive samplings were conducted pre and post fungicide applications.

*Outreach and Dissemination:*
Multiple means of dissemination were utilised to ensure the findings from the project were relayed to the various stakeholders in manner that was both timely but also ensureed provided the information required to change control strategies in a manner that minimised impact. These included presentations at the Teagasc Tillage Conference on an annual basis and the Teagasc Crop Open Days, participation in scientific workshops, symposia and conferences both at national and international levels, articles in the farming press and peer-review journals, regular communication with regulatory authorities including two presentations to personnel in PCRD, dedicated workshops with industry representatives and participation at industry focused meetings/conferences. Following the emergence of SDHI resistance in late 2015, a dedicated conference focusing on septoria control was organised. The conference included national and international speakers. Following the conference dedicated field trial workshops focusing solely on septoria control were organised. The trials series described above was used as the backdrop to this workshop and as such it was held at the three trial sites to ensure as wide a participation as possible.

## 3. Research Achievements/Results

Sub-project 1: Monitoring the Irish *Phytophthora infestans* populations for changes in fungicide and host resistances.

Throughout the life of the project the Irish *P. infestans* population was dominated by three clonal linages (EU_6_A1; EU_8_A1 and EU_13_A2). Strains belonging to the clonal lineages EU_5_A1 and EU_12_A1 were also detected, however this was often sporadic. Further analysis of the EU_8_A1 suggests this lineage may in fact be two closely related lineages. Phenlyamide resistance continued to be detected, predominantly linked to the presence of the linage EU_13_A2, although instances of resistance in EU_8_A1 continued to be detected. Both an agar fungicide sensitivity assay and *in vivo* sensitivity screen confirmed the continued sensitivity of the dominant genotypes to the CAA mandipropamid.

A hybprobe assay was designed to detect potential mutations associated with CAA resistance. Plasmids containing gene fragments with the potential mutations were developed and used as controls in the assay. Sensitivity of the dominant clonal genotypes to the QiI fungicide cyazofamid was also confirmed using an agar plate assay and sequence analysis of their cytochrome *b*. Population analysis of samples obtained from the fungicide field trials (both fungal cultures and lesions stamped onto FTA cards) found no evidence for selection in the local *P. infestans* population following the different fungicide treatment.

As the annual Oak Park *P. infestans* population is a mixture of clonal lineages it was possible to monitor the impacts of cultivar resistances on the population. Collections established from the susceptible varieties contained a mix of the various genotypes, whilst those retrieved from the more resistant cultivars were dominated by strains belonging to the more aggressive clonal lineages EU_6_A1 and EU_13_A2. For the *Rpi-vnt1* gene that has previously demonstrated strong resistance to *P. infestans*, of the strains examined in this study none displayed the ability to overcome what is considered to be an important source of genetic resistance. The detailed effector profile of the Irish strains has been conducted demonstrating that continued monitoring will be required to ensure that virulence doesn't emerge in the in situ population, but equally for the potential migration of novel strains into Ireland that may introduce differing virulence.

Sub-project 2: Monitoring Irish *Zymoseptoria tritici* and *Rhynchosporium commune* populations for changes in fungicide sensitivity and host resistance.

Extensive sampling from commercial fields and fungicide and variety trials were made during the project. From these collections of both *R. commune* and *Z. tritici* isolate collections were established. Using a 454-sequencing assay the mutation G143A in the *R. commune* cytochrome b, known to confer QoI resistance, was detected in a single field in 2013 and in four fields in 2014, ranging in frequency from 2-18% depending on the field. The assay has been optimised to work across multiple platforms including Ion-torrent and Illumina. An additional assay was designed to screen for mutations in the β-tubulin conferring MBC resistance and as expected the mutation E198A known to confer MBC resistance was present at extremely high levels (>95%). During the period covered by the project further reductions in the sensitivity of the Irish *Z. tritici* population to the azole fungicides was detected. These reductions in sensitivity were conferred by multiple mechanisms including the accumulation of CYP51 mutations, CYP51 overexpression conferred by an insert in the CYP51 promoter region and increased efflux activity conferred by multiple inserts in the promoter region of the MgMFS1 efflux pump. Although the frequency of isolates with a large insert (862 and 866 bp in size) in their CYP51 promoter region increased during this same period expression analysis confirmed these inserts had limited impact on sensitivity. KASP markers were developed for the key CYP51 mutations. In 2015 a small number of isolates collected from fungicide trials conducted at Oak Park exhibited reduced sensitivity to the SDHI fungicides. Isolates exhibiting the greatest reduction in sensitivity were found to have the SDHC mutation H152R, whilst those with moderate reductions in sensitivity had the SDHC mutation T79N.

Intensive monitoring in spring 2016 identified further isolates with both mutations, although SDHC-H152R was extremely rare. During the 2016 season rapid selection for SDHC-T79N occurred on specific sites with >65% of isolates collected from the Knockbeg trial site in 2017 having this mutation. Further mutations in the SDHB and SDHD subunits were also found in the national population in 2017. Although the majority of mutations identified only confer moderate reductions in sensitivity, at sites where they are prevalent reduction in efficacy of the SDHI fungicides occurred in the 2017 season. With the exception of fluopyram strong cross-resistance was identified amongst these isolates to the various SDHIs commercially available. Using the 2016 collection the predominance of *Z. tritici* isolates with QoI resistance was also confirmed.

SSR analysis of the *Z. tritici* collection established from the four winter wheat cultivars differing in STB resistance confirmed the Irish *Z. tritici* regularly undergoes sexual reproduction. From this analysis no evidence of specific impacts of cultivar resistance on the Irish *Z. tritici* population was detected. This was further confirmed through whole genome sequencing of a sub-sample of this collection. There was however a minor variety x location specific effect on isolate aggressiveness, with isolates from Stigg obtained from Carlow exhibiting greater levels of disease across the four cultivars inoculated. A total of 10 Avrstb6 haplotypes were identified amongst this isolate collection, with no differences detected between location and cultivar of origin on the frequency of each haplotype. Amongst the collection a single haplotype dominated. The Avrstb6 amplicon sequencing further confirmed the dominance of this haplotype in the wider Z. tritici population, however a greater number of haplotypes were identified, including the wild-type susceptible haplotype.

Sub-project 3: Development of strategies to counter selection of fungicide-resistant and host resistance-breaking strains of *Phytophthora infestans*, *Zymoseptoria tritici* and *Rhynchosporium commune*.

High levels of late blight developed in each trial providing ideal conditions to evaluate the efficacy of each fungicide. With the exception of the multisite mancozeb and the mixture of propamocarb and cymoxanil limited differences were observed between the different fungicides evaluated. Equally no evidence for selection of specific *P. infestans* genotypes from the local population following fungicide treatment was detected.

Even though the cytochrome b mutation G143A was detected in the *R. commune* trial site prior to the trial test applications in post-treatment sampling the mutation was not detected. Although G143A confers *R. commune* resistance to the QoI fungicides and its present has been confirmed in the Irish *R. commune* population its selection appears to be limited or rare.

The impacts of *Z. tritici* SDHI resistance on the efficacy of the SDHI fungicides was evident in the 2017 field trials, with all solo SDHIs only providing moderate control of STB. This control was enhanced with the inclusion of an azole in the mix and further

enhanced with the inclusion of the multisite chlorothalonil. This emphasised the need to ensure all fungicides are applied on time and as part of a mixture including different fungicide groups with activity against STB.

## 4. Impact of the Research

The development of fungicide resistance and host virulence represents a significant threat to the sustainability of the Irish cereal and potato sector. The ability to accurately detect such changes and in a timely manner is critical to ensure strategies can be implemented to mitigate such changes. For each of the pathogens investigated in MonPESC the framework for this has been developed and optimised. This success of this has been evident throughout the project, but is most notable in the contrasting limited detection of G143A in *R. commune*, the initial and subsequent wide spread detection of azole and SDHI resistance in *Z. tritici* and the limited diversity amongst the *Irish P. infestans* population. Combined with field trial evaluations of their impacts MonPESC has provided the basis for the development of control strategies across all three crops of value to all stakeholders. Given the nature of the project all participants ensured the research findings were communicated to relevant stakeholders in a timely manner. As outlined below this took various forms depending on the stakeholder – peer-review papers, farming press, crop walks etc. In the case of *Z. tritici* this culminated in a dedicated conference at which all stakeholders were accommodated (scientific community, agronomy, regulatory, industry, environmental). Given the diversity of platforms through which the findings were disseminated increased exposure for all research institutes and partners and DAFM as the funder occurred amongst stakeholders both at a national and international level.

### 4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research

James Hutton Institute, Scotland:

During his PhD Jeroen Stellingwerf spend a period of time at JHI developing an effector screen for *P. infestans*. This collaboration has continued with a proposal submitted to the Teagasc Research Leaders programme – unfortunately although the proposal was granted the applicant was unable to take up the post.

Aarhus University, Denmark:

As part of his PhD Thies Heick from Aarhus University spend 3 months at Teagasc Oak Park. During this time he investigated the impacts of CYP51 promoter inserts on azole sensitivity. This culminated in a joint peer-review publication. In addition the details on the KASP markers used to screen *Z. tritici* populations for the main CYP51 mutations associated with azole resistance were provided to Aarhus and continue to be used to screen North-European *Z. tritici* populations. These links formed the foundation for the C-IPM project EURO-RES.

Ag. Chemical Industry (including FRAG-UK):
Throughout the life of the projects the research partners regularly provide representatives from the various agri chemical companies and regulators with updates on the sensitivity status of the study pathogens. This included presentations to FRAG-UK, PCRD, Irish and U.K. agronomists and merchants.

European Cereal Disease Control Experts Group:
Dr S Kildea presented findings from the project to the European cereal disease control experts group on an annual basis. This group includes representatives from the U.K., Denmark, France, Germany, Sweden, Norway, Belgium and Finland.

Wider *Zymoseptoria tritici* community:
Throughout the life of the project isolates of *Z. tritici* with varying levels of fungicide resistance/virulence were made available to interested groups when requested. These included INRA, Rothamsted Research, ETZ Zurich.

(ii) Outcomes where new products, technologies and processes were developed and/or adopted

Fungicide screening:
To compliment the traditional *in vitro* and *in vivo* fungicide screening assays a number of molecular assays were developed. These included KASP markers to detect for mutations associated with resistance in *Z. tritici* (cytochrome b mutation G143A, CYP51 mutations D134G, V136C/A, A379G, I381V, S524T), an amplicon sequencing assay for the cytochrome b mutations G143A and the β-tublin mutation E198A and potential mutations at position 140.

Effector screening:
A high throughput amplicon sequencing assay was developed to screen *Z. tritici* field population for the presence of virulent Avrstb6 effectors. Whilst this was originally developed for the ion-torrent sequencing platform it has also been optimised for use on the Illumina platform.

The Pathseq protocol was applied to strains of *P. infestans* isolated from the Irish collection demonstrating the capacity to monitor potential changes in virulence.

(iii) Outcomes with economic potential

Whilst the development of the various techniques to screen and detect resistance/virulence do not have direct economic potential, the ability to develop/adapt control strategies to reflect their detection will maintain disease control of critical pathogens of wheat, barley and potatoes and therefore has indirect economic value.

(iv) Outcomes with national/policy/social/environmental potential

The methods and assays developed are being routinely applied as part of various disease control research projects to study the impacts of different control strategies on the target pathogen populations. It can be anticipated that these will guide future national policies.

**4 (b) Summary of Research Outputs**

(i) Peer-reviewed publications, International Journal/Book chapters.

Dooley H, Shaw MW, Mehenni-Ciz J, Spink J & Kildea S (2016) Detection of the *Zymoseptoria tritici* SDHI-insensitive field isolates carrying the SdhC-H152R and SdhD-R47W substitutions. Pest Management Science 72: 2203-2207 doi.org/10.1002/ps.4269

Phelan S, Barthe MS, Tobin C & Kildea S (2017) Detection of the cytochrome *b* mutation G143A in Irish *Rhynchosporium commune* populations using targeted 454 sequencing. Pest Management Science 73: 1154-1560 doi: 10.1002/ps.4434

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 doi.org/10.1111/ppa.12852

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 doi.org/10.1007/s10658-017-1259-9

Kildea S, Marten-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 doi.org/10.1007/s10658-019-01676-4

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. Irish Journal of Agricultural and Food Research 58: 27-33 doi.org/10.2478/ijafr-2019-0004

(ii) Popular non-scientific publications and abstracts including those presented at conferences

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

Kildea S & Spink J (2013) Controlling Septoria with triazoles. *Irish Farmers Journal Crop Protection Supplement 2013*.

Kildea S (2013) Potato late blight control, a season long battle. *Irish Farmers Journal, May 2013*

Nyongesa M, Phelan S, Wright D, Shaw D, Kildea S, Cooke LR, Griffin D & Mullins E (2013) Evaluating the potential of *Phytophtora infestans* to genetically adapt to the Rpi-blb1 (RB) source of blight resistance. *Proceedings of the fourteenth Euroblight Workshop*

Kildea S, Mehenni-Ciz J, Griffen D & Cooke LR (2013) Sensitivity of Irish *Phytophthora infestans* to the *CAA* fungicide mandipropamide. *Proceedings of the fourteenth Euroblight Workshop*

Kildea S, Mehenni-Ciz J, Spink J & O'Sullivan E (2013) Changes in the frequency of Irish *Mycosphaerella graminicola* CYP51 variants 2006-2011. *Proceedings of the 17th International Rheinhardsbrunn Symposium, p143-144*

Stellingwerf J, Doohan F & Mullins E (2014) Evaluating Avr-vnt1 sequence diversity within Irish *Phytophthora infestans* populations in the presence/absence of Rpi-vnt1. *Oomycete Molecular Genetics Network Meeting 2014*

Kildea S (2014) What does reduced sensitivity mean for field control. *Irish Farmers Journal Crop Protection Supplement 2014*.

Kildea S, Dooley H, Phelan S, Spink J & O'Sullivan (2015) Sensitivity of Irish *Zymoseptoria tritici* populations to the azole fungicides. Resistance 2015, Rothamsted, UK.

Kildea S, Dooley H, Phelan S, Spink J & O'Sullivan E (2016) Sensitivity of Irish *Zymoseptoria tritici* populations to the most commonly applied fungicides. *Proceedings of the Crop Protection in North Britain Conference 2016 P167-168*

Phelan S & Kildea S (2016) Detection of the cytochrome b mutation G143A in the Irish *Rhynchosporium commune* population using targeted 454 sequencing. *Proceedings of the Crop Protection in North Britain Conference 2016 P175-176*

Kildea S, Dooley H, Phelan S, Mehenni-Ciz & Spink J (2016) Developing fungicide control programmes for septoria tritici blotch in Irish winter wheat crops. *Proceedings of the 18th International Rheinhardsbrunn Symposium, p171-174*

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

(iii) National Report

None generated

(iv) Workshops/seminars at which results were presented

In 2017 a national Septoria Conference was organised as part of the MonPESC project. The conference was held at the Dunboyne Castle Conference Centre on March 23rd. A total of seven full presentations were presented covering all aspects of STB control, with additional industry presentations from representatives of each of the main agri-chemical and seed companies. The conference was attended by over 150 people.

In addition the various MonPESC partners presented research findings at various meetings, conferences and workshops as detailed below.

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| Kildea S | European Cereal Disease Control Extension Meeting 2013 - Germany | To discuss cereal disease control experiences during 2012 | 07/02/13 |
| Mullins E | Euroblight 2013, Limasol, Cyprus | Evaluating the potential of *Phyhthora infestans* to genetically adapt to the Rbi-blb1 transgne | 13/05/13 |
| Kildea S | Annual Meeting of the Irish Fungal Society – Maynooth – Invited Speaker | Ten years of monitoring Irish *Mycosphaerella graminicola* for fungicide resistance | 20/06/13 |
| Kildea S | Teagasc Crops Open Day 2013 | Winter Wheat Disease Control | 26/06/13 |
| Cooke LR | Society of Irish Plant Pathologists Autumn Meeting 2013 | Controlling late blight disease of potato | 02/09/13 |
| Cooke, LR, | Syngenta Belfast Technical Conference 2013 | To give presentation "Update on potato blight and its control" and meet NI merchants, growers | 11/02/14 |
| Cooke, LR, | Syngenta Belfast Technical Conference 2013 | Update on potato blight and its control | 11/02/14 |
| Kildea S, Glynn L | Teagasc Tillage Conference | Cereal Disease Control | 30/01/14 |
| Kildea S, | European Cereal Disease | Cereal Disease Control | 20/02/14 |

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| | Control Extension Meeting 2014 - Denmark | | |
| Kildea, S | Teagasc Advisory In service – Teagasc, Oak Park, Carlow | Cereal Disease Control | 01/03/14 |
| Kildea, S | Presentation to TCD students – Teagasc Oak Park, Carlow | Ten years of monitoring Irish cereal pathogens for fungicide resistance | 01/03/14 |
| Kildea, S | European Cereal Disease Control Extension Tour 2014 – Oak Park Carlow | Trial Demonstrations | 11/07/14 |
| Kildea S | Teagasc Tillage Conference | Cereal Disease control for 2015 | 29/01/15 |
| Kildea S | European Cereal Disease Control Extension Meeting 2015 –France | Cereal Disease Control | 19/02/15 |
| Kildea S | Presentation to TCD students – Teagasc Oak Park | Cereal Disease Control | 30/03/15 |
| Kildea S | Presentation to Irish Agchem industry representatives – Teagasc, Oak Park, Carlow | Fungicide Resistance Up-dates | 12/05/15 |
| Kildea | Presentation to PCRD, DAFM Labs, Cellbridge, Kildare | Fungicide Resistance Up-dates | 26/05/15 |
| Black L, Cooke LR, Young GK | Agronomy and Business Management Conference for Cereal Growers | Poster Presentation: Mon:PESC Monitoring Pathogen Evolution for Sustainable Cropping. | 17/01/15 |
| Black L and White E | 5 growers meetings in Northern Ireland (June - July 2015) | Hosting variety trial visits - discussion of the MonPESC project with advisors, growers and seed merchants. | June and July 2015 |
| Phelan S | IPSAM 2015 | Monitoring the Irish the Rhynchosporium commune population to detect the G143A mutation on the cyctochrome b using targeted 454 sequencing | 11/05/15 |

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| Phelan S | Presentation to Irish Agchem industry representatives, Teagasc, Oak Park, Carlow | Fungicide Resistance Up-dates | 12/05/15 |
| Phelan S | Presentation to PCRD, DAFM Labs, Cellbridge, Kildare | Fungicide Resistance Up-dates | 26/05/15 |
| Phelan S | Irish Fungal Society 2015 - TCD | Monitoring the Irish the Rhynchosporium commune population to detect the G143A mutation on the cyctochrome b using targeted 454 sequencing | 11/06/15<br>12 /06/15 |
| Phelan S | Society of Irish Plant Pathologists Meeting 2016, Hillsborough, N. Ireland | Detection of the cytochrome *b* mutation G143A in Irish *Rhynchosporium commune* populations using targeted 454 sequencing | 13/10/16<br>14/10/16 |
| Kildea S | Society of Irish Plant Pathologists Meeting 2016, Hillsborough, N. Ireland | Effect of fungicides on local P. infestans populations | 13/10/16<br>14/10/16 |
| Kildea S | Association of Independent Crop Consultants, U.K. – Invited Speaker | Controlling septoria tritici blotch | 13/01/16 |
| Kildea S | National Tillage Conference | Wheat Disease Control and Resistance Issues | 28/01/16 |
| Kildea S | BASF Technical Meeting, Kilkenny – Invited Speaker | Barley Disease Control - 2016 | 11/02/16 |
| Kildea S | European Cereal Disease Control Extension Meeting 2016 - Sweden | Wheat Disease Control and Resistance Issues | 18/02/16<br>19/02/16 |
| Kildea S | Fungicide Resistance Action Group - UK | Zymoseptoria tritici Irish sensitivity up-date | 15/03/16 |
| Kildea S | Presentation to PCRD, DAFM Labs, Cellbridge, Kildare | SDHI Resistance Update | 18/01/16 |

| | | | |
|---|---|---|---|
| Kildea S | Presentation to TCD students, Teagasc, Oak Park, Carlow | Controlling Cereal Diseases to maximise profit | 04/04/16 |
| Kildea S | Presentation to Tralee IT students, Teagasc, Oak Park, Carlow | Controlling Cereal Diseases to maximise profit | 07/04/16 |
| Kildea S | Presentation to UCD students, Teagasc, Oak Park, Carlow | Fungicide Resistance, winning the battle but losing the war | 21/10/16 |
| Welch T | 9th International SymposiumD on septoria diseases of cereals – Paris, France | Monitoring genetic response of Zymoseptoria tritici populations to host resistance | 07/04/16<br>09/04/16 |
| Black L | North West Growers' Meeting, Limavady, Derry, N. Ireland | Update on fungicide resistance | 04/07/16 |
| Black L | CAFRE Growers' Meeting, Crossnacreevy, Antrim, N. Ireland | Update on fungicide resistance | 05/07/16 |
| Black L | CAFRE Growers' Meeting, Strabane, Tyrone, N. Ireland | Update on fungicide resistance | 19/07/16 |
| Kildea S | Irish Fungal Society Annual Meeting 2017 – LIT, Limerick | Poster on Azole Resistance in Zymoseptoria tritici | 15/06/17 |
| Phelan S | Irish Fungal Society Annual Meeting 2017, LIT, Limerick | Presentation Impact of fungicides on P. infestans populations | 15/06/17 |
| Welch T | Irish Fungal Society Annual Meeting 2017, LIT, Limerick | Poster on impact of host on Zymoseptoria tritici populations | 15/06/17 |
| Phelan S | Euroblight 2017, Aarhus, Denmark | Presentation Impact of fungicides on P. infestans populations | 14/05/17 |
| Stellingwerf J | Euroblight 2017, Aarhus, Denmark | Presentation Impact of host on P. infestans populations | 14/05/17 |
| Welch T | Zymoseptoria titici Community Meeting Kiel 2017 | Impact of variety on AvrStb6 | 05/09/17 |

(v) Intellectual Property applications/licences/patents

Not applicable

(vi) Other

Research findings were presented at the bi-annual Teagasc Crops Open Day, and in 2017 at dedicated Septoria field trial walks. These were organised in association with the Teagasc Tillage Specialists team.

| | | | |
|---|---|---|---|
| Kildea S | Septoria Crop Walk - Carlow | Update on field control / Resistance | 07/07/17 |
| Kildea S | Septoria Crop Walk - Meath | Update on field control / Resistance | 03/07/17 |
| Kildea S | Septoria Crop Walk - Cork | Update on field control / Resistance | 06/07/17 |
| Kildea S | Crops Open Day 2017 | Field Control | 28/06/17 |
| Phelan S | Crops Open Day 2017 | Fungicide sensitivity | 28/06/17 |
| Kildea S | Teagasc Crops Open Day 2013 | Winter Wheat Disease Control | 26/06/13 |

## 5. Scientists trained by Project

Total Number of PhD theses: 2

Thomas Welch, University College Dublin, September 2018
Title: Monitoring changes in Irish *Zymoseptoria tritici* populations in response to host resistances

Jeroen Stellingwerf, University College Dublin, September 2017
Title: Investigating the potential adaptation of *Phytophthora infestans* in Ireland against its primary host *Solanum tuberosum*.

Total Number of Masters theses: 0

## 6. Permanent Researchers

| Institution Name | Number of Permanent staff contributing to project | Total Time contribution (person years) |
|---|---|---|
| Teagasc | 3 | 2.23 |
| UCD | 3 | 0.6 |
| AFBI | 3 | 0.65 |
| **Total** | | |

## 7. Researchers Funded by DAFM

| Type of Researcher | Number | Total Time contribution (person years) |
|---|---|---|
| Post Doctorates/Contract Researchers | 1 | 2.763 |
| PhD students | 2 | 7.917 (4 & 3.917) |
| Masters students | 0 | 0 |
| Temporary researchers | 4 | 1.24 |
| Other | 1 | 0.250 |
| **Total** | | |

## 8. Involvement in Agri Food Graduate Development Programme

None

## 9. Project Expenditure

| | |
|---|---|
| Total expenditure of the project: | €752,573.12 |
| Total Award by DAFM: | €759,827.88 |
| Other sources of funding including benefit in kind and/or cash contribution(specify): | €0 |

Breakdown of Total Expenditure

| Category | Teagasc | UCD | AFBI | Total |
|---|---|---|---|---|
| Contract staff | 0 | 0 | 0 | |
| Temporary staff | 3446.07 | 0 | 20,339.92 | 23,785.99 |
| Post doctorates | 145,245.68 | 0 | 0 | 145,245.68 |
| Post graduates | 0 | 175,149.79 | 0 | 17,149.79 |
| Consumables | 155,263.96 | 42,488.87 | 3,813.70 | 201,566.53 |
| Travel and subsistence | 10,900.50 | 9,220.46 | 327.63 | 20,448.59 |
| Sub total | | | | |
| Durable equipment | 0 | 0 | 0 | 0 |
| Other | 14,842.53 | 0 | 0 | 14,842.53 |
| Overheads | 94,456.86 | 68,057.74 | 7,344.38 | 169,858.97 |
| **Total** | **425,830.64** | **294,916.86** | **31,825.63** | **752,573.12** |

## 10. Leveraging

The various partners involved in MonPESC have been successful in using the project to leverage funding for additional projects. These have included funding national funding from DAFM, Teagasc and SFI including projects continuing the monitoring and detection of fungicide resistance and/or virulence (Teagasc: Monitoring pathogen populations, Designing future disease control; DAFM: SCOPE, EPIC, BioCrop, VICCI; SFI: ReWIZ), international funding (H2020 Marie Curie European Training Networks: CerealPath; C-IPM ERANET: EUROWHEAT: Fungicide Resistance Network), industry funding (both cereal breeding companies & agrichemical manufactures).

## 11. Future Strategies

Phytopathological collections:

During MonPESC extensive collections of *P. infestans*, *Z. tritici* and *R. commune* were established. These have been maintained either on FTA cards (*P. infestans*) or as live cultures in -80°C (*Z. tritici* & *R. commune*). These will continue to be utilised to monitor changes in either virulence or fungicide resistance to current and future molecules. In addition a single spore collection of *Z. tritici* has been generated spanning the years 2005-2018 and is being used to monitor in detail changes in *Z. tritici* during this time period. The isolates which have been sequenced will be used for virulence screens and fungicide sensitivity screening. The pooled DNA collections for both *Z. tritici* and *R. commune* continue to be used as part of a number of projects to monitor fungicide resistance. The 2017 pre-treated *Z. tritici* field collection is being used as control isolates in the development of a sequencing assay capturing the genes involved in fungicide resistance.

Similarly isolates from the *Z. tritici* collection have been provided to researchers in Rothamsted, INRAE, Aarhus University, SLU and agrichemical manufactures for use in optimisation of detection assays and/or evaluation of fungicide efficacy. As per the original proposal Teagasc will continue to make these isolates available to researchers upon request.

Molecular screening assays:
All amplicon sequencing assays have been adapted to ensure compatibility with the Illumina sequencing platform, with additional assays for the detection of mutations associated with SDHI resistance in *Z. tritici* developed. These assays now form a key method in screening for fungicide resistance in *Z. tritici* and *R. commune* and are utilised as part of the development and optimisation of control programmes for both pathogens. The skills and knowledge developed will also continue to be applied to additional cereal and potato pathogens e.g. *Ramularia colli-cygni*. The KASP markers developed for *Z. tritici* continue to be used across a number of research programmes and Teagasc continue to ensure access to the sequence information for these are made available.

Disease control programmes:
Following the loss of key fungicides and/or the breakdown in resistance genes increased pressure will be placed upon the remaining fungicides and resistance genes. The development of control programmes that reduce these pressures will be vital to the success of future disease control measures. The various resources, techniques and knowledge developed in MonPESC will be key to the development of future control strategies.

## 12. Consent to Publish Final Report on the DAFM Website and/or Through Other Dissemination channels

I consent to this report being made available to the public, through the Department's website and other dissemination channels.

Yes X

## 13. Declaration

I declare that the information contained in this final report is complete and true to the best of my knowledge and belief.

Signed:  Project Coordinator

Date: 30.09.2020