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Monitoring fungicide sensitivity in the major pathogens of Irish cereal crops

Key external stakeholders:

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

Practical implications for stakeholders:

The development and spread of fungicide resistance in the main diseases of arable crops represents a significant and increasing problem for the Irish arable sector. Careful consideration of all available means of controlling arable pathogens should be taken to reduce the pressure on available fungicide chemistry.

- The loss of the key fungicides whether through changes in the regulations or development of resistance in the target pathogens represents a serious threat to the viability of the Irish arable sector.
- The Irish *Zymoseptoria tritici* population now contains strains exhibiting resistance to the QoIs, azole and SDHI fungicides.
- Irish populations of both *Microdochium nivale* and *M. majus* are dominated by strains with the G143A mutation conferring resistance to the QoI fungicides, which should not be relied upon for control of these pathogens.
- Resistances to at least one of the main fungicides used for control of diseases in barley have been recorded amongst the major barley pathogens. However a diversity of sensitivity still exists and barley fungicide programmes should incorporate a range of fungicide groups to maximise this diversity.

Main results:

The sensitivity of the major pathogens of Irish cereals to the most commonly used fungicides was monitored using a combination of phenotypic and molecular analysis. Resistance to one or more of the major fungicides, with the exception of the multisite fungicides is now present in all the major pathogens of wheat and barley. In the case of *Zymoseptoria tritici*, cause of septoria tritici blotch of wheat, resistance has now been detected to the QoIs, azoles and SDHIs. The frequency of resistance to both the QoIs and azoles is now sufficiently high in the population to adversely affect the efficacy of both groups of fungicides under field conditions. A range of sensitivities exist in the major diseases of barley and as such fungicide programmes in barley should utilise a range of fungicide groups.

Opportunity / Benefit:

Although fungicide resistance and increased regulations restrict the effectiveness and availability of the most commonly applied fungicides, knowledge of the fungicide sensitivity of the major arable pathogens is being utilized to design control strategies that provide effective disease control while minimizing fungicide resistance emergence and selection.

Collaborating Institutions:

Scottish Rural College (SRUC), BASF

Contact Steven Kildea Email: Stephen.kildea@teagasc.ie.

Teagasc project team: Dr. Steven Kildea (PI)
John Spink
Niamh Kelly (Walsh Fellow – Teagasc funding)
Marta Jankoswka (Walsh Fellow – BASF funding)

External collaborators: Prof. Fiona Burnett (SRUC);
BASF

1. Project background:

Due to the mild climatic conditions often experienced in Ireland, fungal diseases can reduce potential cereal yields by anything up to 40%. To prevent such losses fungicides are currently relied upon. Whilst production systems continue to rely heavily upon fungicides for disease control the potential for fungicide resistance to develop in the major pathogens of wheat and barley represents a serious threat to the sustainability of arable production. It is therefore essential to continually monitor the fungicide sensitivity status of the major pathogens. To maximize available resources such monitoring must combine phenotypic sensitivity assays with detailed molecular studies.

In combination with routine monitoring it is essential to determine what these changes in sensitivity mean for disease control under field conditions and if strategies can be implemented that reduce the selection and spread of resistance. Such strategies may include the mixing or alternation of different modes of action and/or reducing the rates at which fungicides are applied.

2. Questions addressed by the project:

- What is the current fungicide sensitivity status of the major pathogens of wheat and barley?
- What fungicide resistance mechanisms confer fungicide resistance in Irish wheat and barley pathogens?
- What impact does the presence of resistant strains have on the efficacy of fungicides against the major foliar pathogens of wheat and barley?
- Can strategies taking account of the fungicide sensitivity of the pathogen's population be put in place that maintain disease control but limit the spread of resistance?

3. The experimental studies:

Collections of *Zymoseptoria tritici*, *Rhynchosporium commune*, *Pyrenopora teres*, *Micodochium nivale* and *M. majus* were established from commercial crops and field trials in 2012-2016. Sensitivity of the collections to a range of fungicides including, representative fungicides belonging to the azoles, SDHIs, MBCs and QoIs were determined using either a microtiter plate assay or an agar plate assay. In both instances the sensitivity was recorded as either the minimum inhibitive concentration (MIC) or effective concentration to reduce growth by 50% (EC50). Where possible, the impact of target site alterations and/or alternative resistance mechanisms were investigated by sequencing the respective target sites in isolates representing the various sensitivity profiles detected. Where mutations associated with fungicide resistance were identified molecular assays were developed to aid detection in field populations. These included KASP assays for six of the key *CYP51* mutations involved in *Z. tritici* azole resistance, a PCR-RFLP assay to detect the cytochrome *b* mutation G143A involved in *M. nivale / M. majus* resistance to the QoIs and a 454-sequencing assay to simultaneously detect the cytochrome *b* mutations F129L and G137R involved in *P. teres* resistance to the QoI fungicides. These assays were subsequently used to both screen the wider pathogen population and to determine both the impact of resistance on disease control and the effectiveness of fungicide anti-resistance strategies.

To determine the impact of resistance on fungicide efficacy field trials were conducted each season. The sensitivity of the target pathogen prior and post treatment was determined using the various assays described above. In the case of SDHI resistance in *Z. tritici*, *in planta* experiments were conducted to determine potential impacts the different resistances detected in the Irish population may have on field efficacy. Similarly field trials investigating the effectiveness of anti-resistance strategies (dose, mixing/ alternating different modes of action) were conducted for *Z. tritici*, *R. commune* and *P. teres*. Further

Contact Steven Kildea Email: Stephen.kildea@teagasc.ie.

investigations into the impact of mixing azoles on the diversity of *CYP51* within *Z. tritici* post treatment as previously described by Dooley et al. (2016) were also conducted.

Dooley H, Shaw MW, Spink J and Kildea S (2016) Effect of azole fungicide mixtures, alternations and dose on azole sensitivity in the wheat pathogen *Zymoseptoria tritici*. *Plant Pathology 65:124-136*

4. Main results:

Strains exhibiting resistance to one or more fungicide modes of action are now present in the Irish population of each of the major pathogens of wheat and barley.

In the *Z. tritici* population resistance has now being detected to all the major systemic fungicides, with reductions in efficacy recorded at field level for both the QoIs and azoles, and expectant to occur for the SDHIs based on *in planta* data. Resistance to the QoIs is conferred by the G143A mutations and is widespread at extremely high frequencies in the Irish *Z. tritici* population. A combination of complex *CYP51* mutations, and/or CYP51 overexpression, and/or efflux overexpression is also present at varying frequencies in the population. The presence of SDHI resistance, conferred by the mutations C-T79N or C-H152R was first detected in the Irish *Z. tritici* population in 2015 and subsequently throughout the 2016 season. Alternation of the specific azole fungicide within the fungicide programme does select for different CYP51 mutations and as such it is recommended to include as much azole diversity in a fungicide programme as is possible – ensuring alternation is between the different groups of azoles. Even in the presence of high levels of QoI resistance, pyraclostrobin continues to provide moderate levels of *Z. tritici* control. As the mixing of an SDHI and QoI did not significantly improve *Z. tritici* control or increase yields, urther analysis is required to determine if the inclusion of a QoI with an SDHI reduced selection for SDHI resistance.

While strains with a functional CYP51a were detected in the population, the Irish *R. commune* population remains moderately sensitive to the azole fungicides. Unlike previously reported by Hawkins et al (2014) these strains did not exhibit reduced azole sensitivity compared to those without a functional CYP51a. As previously reported by Phelan et al. (2016) QoI resistance remains sporadic if detectable. Combining different effective modes of action provides the most efficient control of *R. commune* under field conditions.

Resistance to specific QoI fungicides, conferred by the cytochrome *b* mutation F129L was detected at low levels in the Irish *P. teres* population. Field trials conducted in 2014 and 2015 confirmed that although initially low, selection for the mutation did occur post QoI treatment, with the efficacy of specific QoIs impacted. The first instances of *P. teres* resistance to the SDHIs were detected in samples from Northern Ireland in 2014. The frequency of resistance was extremely low and the mutations detected (D-D145G) are believe to provide only moderate resistance.

High levels of ear blight in wheat and barley were recorded in 2012. These infections were predominantly caused by both *M. nivale* or *M. majus*. Resistance to the QoIs was extremely high in both populations and the efficacy of the QoIs in the final fungicide application for ear blight is expected to be dramatically reduced and is no longer recommended. Resistance to the MBC fungicides was detected in both pathogens, however the frequency of resistance differed significantly with high levels detected in *M. majus* collection and low levels in *M. nivale* collection.

Hawkins NJ, Cools HJ, Sierotzki H, Shaw, MW, Knogge W, Kelly S. and Fraaije BA (2014) Paralog re-emergence: a novel, historically contingentmechanism in the evolution of antimicrobial resistance. *Molecular Biology and Evolution, 31 1793-1802*

5. Opportunity/Benefit:

The collections established represent contemporary field populations of the most economically destructive pathogens of Irish cereal crops and are being used to determine how sensitivities are changing over time and to evaluate future modes of action, including biologicals. The assays designed are routinely used to determine sensitivity of trial sites and commercial crops prior and post fungicide application.

6. Dissemination:

Knowledge Transfer events:

Contact Steven Kildea Email: Stephen.kildea@teagasc.ie.

Septoria Crop Walks: Carlow (17th June 2014), Meath (19th June 2014), Cork (20th June 2014)

Tillage Crops Open Day, Oak Park Carlow: 26th June 2013; 24th June 2015

Kildea S and Glynn (2012) Cereal fungicide sensitivity and performance. *National Tillage Conference 2012 P66-79*

Kildea S (2013) Fungicide sensitivity and disease control. *National Tillage Conference 2013 P53-60*

Kildea S (2014) Cereal disease control. *National Tillage Conference 2014 P91-102*

Kildea S and Glynn L (2015) Cereal Disease control for 2015. *National Tillage Conference 2015 P35-46*

Kildea S (2016) Wheat disease control and resistance issues. *National Tillage Conference 2016 P39-46*

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 (accepted)*

Jess S, Kildea S, Moody A, Rennick G, Murchie, Cooke LR (2014) European Union policy on pesticides: implications for agriculture in Ireland. *Pest Management Science 70, 1646-1654*

Conference Papers:

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

Kildea S, Dooley H, Phelan S, Mehenni-Ciz J, Spink J (2017) Developing fungicide control programmes for septoria tritici blotch in Irish winter wheat crops. *In: Deising HB; Fraaije B; Mehl A; Oerke EC; Sierotzki H; Stammler G (Eds), "Modern Fungicides and Antifungal Compounds", VIII, P171-174*

Kildea S, Mehenni-Ciz J, Spink J and O'Sullivan E (2014) Changes in the frequency of Irish *Mycosphaerella graminicola* CYP51 vaiants 2006-2007. *In: Deising HB; Fraaije B; Mehl A; Oerke EC; Sierotzki H; Stammler G (Eds), "Modern Fungicides and Antifungal Compounds",VII, P143-144.*

Popular publications:

Kildea S (2016) SDHI Resistance – The Irish Experience. *Crop Protection Supplement 2016*

Kildea S and Spink J (2014) Implications of increased pesticide regulations on cereal disease control in Ireland. *TResearch 8. P30-31*

Kildea S and Connolly C (2014) Fungicide resistance in head blight. *TResearch 9. P26-27*

Kildea S (2015) Understanding Disease Control in Cereal Crops. *Crop Protection Supplement 2015 P8-10*

Thesis:

Jankoswka M (2016) Quantifying synergy between the strobilurins and SDHIs in control of septoria tritici blotch. MPhil in Evolutionary Biology, University of Edinburgh.

7. Compiled by: Dr. Steven Kildea

Contact Steven Kildea Email: Stephen.kildea@teagasc.ie.