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Screening for QoI resistance in UK populations of *Rhynchosporium secalis*

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This is the final report of an 18 month project (RD-2009-3645) which started in June 2009. The work was funded by a contract for £55,257 from HGCA.

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1. ABSTRACT

The development of strobilurin (QoI (quinone outside inhibitors)) fungicide resistance is now widespread in many fungal plant pathogens. However, this group of fungicides is still widely used to control *Rhynchosporium secalis* in winter and spring barley crops across the UK and have so far retained their efficacy. Resistance to this fungicide group in the majority of fungal pathogens is the result of a single point mutation found at either codon 143, 129 or 137 in the cytochrome *b* gene. During 2008, routine screening of the *R. secalis* populations by BASF discovered that some isolates in northern France had developed a mutation at codon 143.

This research examined the UK population of *R. secalis* over two growing seasons (2009-2010) for the occurrence of the mutations causing resistance to QoI fungicides with the combined use of molecular techniques and 96 well plate fungicide sensitivity assays During this project, a high throughput pyrosequencing assay was developed to examine populations directly from field samples. This coupled with the fungicide bioassays and a simple Polymerase Chain Reaction Random Amplified Length Polymorphism (PCR-RFLP), (a simple enzyme methodology that cuts DNA into small parts only if mutations causing fungicide resistance are present) indicated that the UK *R. secalis* population remained fully sensitive to QoI fungicides during the period of this project (2008-2010) with no declines in efficacy. However, this does not mean that resistance will not develop in the coming years and ongoing independent screening will be required to monitor the situation in the coming growing seasons.

2. SUMMARY

2.1. Introduction

Leaf blotch caused by *Rhynchosporium secalis* is the most economically important disease of barley in the United Kingdom. The estimate for barley production in the UK is 5.7 million tonnes, which has a value of £770 million at £135/tonne (Defra, 2011 based on the 5-year average data). The losses from disease in winter barley were estimated to be £5m and of this £2.57m resulted from that caused by *R. secalis* (Defra, 2011 data).

The development of strobilurin (QoI (quinone outside inhibitors)) fungicide resistance is now widespread in many fungal plant pathogens. However, this group of fungicides is still widely used to control *R. secalis* in winter and spring barley crops across the UK and have so far retained their efficacy. Resistance to this fungicide group in the majority of fungal pathogens is the result of a single point mutation found at either codon 143, 129 or 137 in the cytochrome *b* gene.

During 2008, routine screening of the *R. secalis* populations by BASF discovered that some isolates in northern France had developed a mutation at codon 143 (FRAC www.frac.info). The presence of this mutation was reported to cause *R. secalis* to become completely resistant to QoI fungicides. However, despite the development of the first field isolates of *R. secalis* resistant to QoI fungicides, the occurrence of the mutation was found at a low level and from only one location.

Once resistance develops in a crop it can be expected to develop quite rapidly thereafter, providing the resistance is stable. The risk to the UK from QoI fungicide resistance in *R. secalis* is considered to be high, due to the seed borne nature of this disease and the ability of the pathogen to develop stable resistance, thus putting further pressure on the sterol demethylation inhibitors (DMI) or azole groups, which have already been shown to be less effective than in the past (Oxley and Burnett, 2010).

Screening for fungicide resistance is not widely performed in the UK and a very limited amount is currently available in the public domain. Less than 12 isolates of *R. secalis* were tested by BASF for the whole of the UK in 2008, despite the widespread use of QoI fungicides in barley disease control. Therefore, this project was considered vital to assess independently the current situation of QoI resistance in *R. secalis*.

2.2. Materials and methods

Molecular-based techniques were used in this project to screen single spore isolates of the fungus from a wide variety of locations across the UK including the south-west of England, south-eastern England, East Anglia, Scotland and Northern Ireland. Single spore isolates are the strains of the pathogen grown from a single piece of the fungus, either a single spore or mycelium fragment, these individuals isolates are genetically distinct and uniform making them suitable to check for their resistance status. The molecular methods enabled accurate screening for single point mutations that can cause QoI fungicide resistance. In total 110 individual isolates had the target cytochrome *b* gene partially sequenced to check for the presence of any nucleotide changes and over 300 isolates were checked for the G143A mutation using a Polymerase Chain Reaction Random Amplified Length Polymorphism (PCR–RFLP). This simple test allows the screening of isolates for the presence of known mutations following the digestion with a restriction enzyme that only digests to the presence of the mutation causing QoI fungicide resistance. A similar number of field samples were also checked using the newly developed pyrosequencing assay. These techniques are all widely used in the agrochemical industry for screening for resistance.

This project also widely tested isolates of the fungus using a more traditional fungicide bioassay to calculate the half-maximal effective concentration EC50 values. These data were then compared to the resistant isolates (from northern France) which were used as a control in the bioassays.

2.3. Results

The range of EC_{50} values in the sensitive population was found to be between 0.007 to 0.35 µg/ml, whereas the resistant isolates consistently produced an EC_{50} value of around 6.4 µg/ml, showing that the occurrence of the G143A mutation had a dramatic effect on fungicide performance through a single change in the target cytochrome *b* gene changing a glycine to alanine at codon 143 (G143A). The combined screening using both conventional bioassays and a high throughput-sequencing assay has allowed many more samples to be tested during this project than originally proposed. However, the results of this study showed that no mutations causing QoI fungicide were detected in any UK samples during the project duration.

2.4. Conclusions

The mutation causing resistance has not been found in the UK, during the lifetime of this project. This is probably either because there are very low numbers of resistant strains in the UK or because currently the mutations are not present. However, the data generated do show some background variation in the EC_{50} values generated. This means that the efficacy of this fungicide group is variable and may suggest that resistance could occur in the future, if a single point mutation could arise. However, the EC_{50} values that have been generated for this project are all within the expected range for full field control. While the outcomes of this project are positive for the continued use of QoI fungicides, it should be noted that resistance has been shown to occur in a natural population and the reasons that this resistance has not spread are currently not clear. Therefore, the future of QoI fungicides for the widespread control of *R. secalis* may be in doubt in the future as the selection pressure of fungicide use could continue to drive the evolution towards a fully resistant population.