Wheat Powdery Mildew – Using New Fungicide Resistance Detection Methodologies to Stay Ahead of the Game

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Key messages

- Wheat powdery mildew caused by the fungal pathogen *Blumeria graminis* f. sp. *tritici* (*Bgt)* was a surprising threat to the West Australian (WA) wheat industry in the 2015 cropping season, with many growers reporting some degree of infection.
- Recently *Cyp51* mutations (Y136F and S509T), have been identified in barley powdery mildew isolates exhibiting some degree of resistance to many triazole fungicides, particularly tebuconazole. The Y136F mutation, although having minimal effect in the field, acts as a gateway mutation, initially present in isolates who mutate further translating to field level resistance.
- Digital PCR (dPCR) is a new technology in fungicide resistance mutation detection that can be used to screen several million fungal spores simultaneously.
- For the first time, dPCR was used to screen for the Y136F gateway mutation in *Bgt.* The mutation was found in significant numbers in the east of Australia but not in WA.

Aims

Develop a high-throughput, cost effective, molecular method to screen samples of *Blumeria graminis* f. sp. *tritici* (*Bgt)* the causal agent of wheat powdery mildew, for *Cyp51* mutations shown to be associated with resistance to triazole fungicides.

Background

Powdery mildew has plagued Western Australian (WA) barley growers for much of the last decade. Until recently wheat powdery mildew, although present in WA crops, had only caused negligible loses to growers. That was until last season, where favourable conditions saw *Bgt* spread rapidly throughout most of the WA wheatbelt, catching many wheat growers by surprise.

Fungicide resistance in the barley powdery mildew pathogen *Blumeria graminis* f. sp. *hordei* (*Bgh*) has been known in WA since 2011, where the combination of wide scale planting of susceptible cultivars coupled with the prolonged use of predominantly tebuconazole based fungicides led to the dominance of a triazole resistant phenotype (Tucker, Lopez-Ruiz et al. 2014). In *Bgh* two mutations were discovered in the triazole target gene, *Cyp51*. These mutations, Y136F and S509T, were shown to have little impact on fungicide efficacy when present as single changes in an isolate. The biggest increase in resistance was shown in isolates that possessed both *Cyp51* changes, particularly in relation to tebuconazole. To date, the S509T mutation has never been found in *Bgh* isolates lacking Y136F. Interestingly studies in other fungal crop

pathogens, including *Bgh,* have shown that Y136F acts as a precursor or gateway to subsequent *Cyp51* mutations that tend to accumulate in isolates with increasing levels of triazole resistance.

In comparison, wheat powdery mildew has remained a minor pathogen in Australia leaving many growers and agronomists to focus on minimising the impact of the more prevalent and damaging diseases such as rust and tan spot (Murray and Brennan). Prior to the wheat powdery mildew epidemic of 2015, only a small collection of *Bgt* isolates had been screened for both reductions in triazole sensitivity and the presence of *Cyp51* changes. Neither mutations in *Cyp51* nor shifts in triazole sensitivity were detected.

Conventionally, researchers have combined traditional phenotyping and genotyping techniques to detect fungicide resistance in fungal crop pathogens. These techniques although effective, require extensive labour and materials and can take weeks, if not up to a month, for biotrophic pathogens such as the powdery mildews. A quick turnaround in the time taken to relay any confirmed cases of resistance to other researchers, advisers and growers, plays a pivotal role in implementing on farm and state wide practices for disease management in the current season and beyond. Therefore, a high-throughput, molecular method was required in which fungicide resistance results can be relayed in a matter of days not weeks or months, to implement rapid management practices if resistance were detected in *Bgt.*

Method

An Introduction to Digital PCR technology

Digital Polymerase Chain Reaction (dPCR) is a molecular technique that that brings mutation detection by PCR (Polymerase Chain Reaction) into the digital age. Similar to real-time quantitative PCR, dPCR carries out a single reaction within an individual tube. However the difference with dPCR is that the sample is separated out on a silicon chip into up to 10 million partitions, where a PCR reaction is carried out in each individual partition. In practice, dPCR requires utilisation of fluorescently labelled probes that have been designed to bind exclusively to mutations of interest. In each partitioned reaction, the binding of a probe to the target mutation indicates a positive reaction resulting in the emission of a fluorescent fluorophore. The fluorescent fluorophore is digitally detected and reported. In this manner, up to 10 million individual PCR reactions are performed simultaneously and each positive fluorescent signal can be used to quantify the frequency of target mutation in a single sample. This type of technology has been used in the medical field for over 2 decades (Sykes, Neoh et al. 1992) but until now has not yet to be applied in the detection of fungicide resistance mutations in fungal crop pathogens. Fluorescently labelled dPCR probes were designed to detect the gateway Y136F mutation in *Bgt,* based on the homologous mutations in the highly similar barley powdery mildew pathogen

Sampling

Samples of wheat leaves infected with powdery mildew were collected from sites in Australia, covering a range of agricultural zones around the WA wheatbelt and several sites from the eastern states. Samples

collected in WA were taken from Geraldton in the North to as far south as Esperance with many samples being submitted from within a 200km radius of the Perth metropolitan area. Samples from outside WA included those collected in New South Wales, Tasmania and South Australia. Leaf tissue from the same site were pooled according to plot and/or fungicide treatment for analysis.

Results

Perhaps the greatest advantage of dPCR over genotyping by conventional PCR, is the assay's ability to quantify the absolute number of mutant DNA sequences within each sample. Traditionally, once a fungal sample has been taken from the field it must be purified to ensure that each conventional PCR reaction contains DNA from only a single isolate, an often long and laborious task. Digital PCR eliminates this process. Whole infected tissue samples destined for dPCR analysis are first mechanically ground and then fed into an automated process to extract the total leaf and fungal DNA present. The resulting mixed DNA sample is then loaded onto a dPCR silicon chip and the total amount of mutant DNA quantified.

Over the 2015 season many wheat samples were received infected with powdery mildew. Tissue from the same site and treatment were assayed together and total DNA was extracted from each pooled sample. In total 71 individual dPCR assays were run, screening for the presence of the Y136F. These assays included tissue collected from 53 WA fields and 18 from the eastern states of Australia incorporating sample sites in New South Wales, Tasmania and South Australia.. The precursor Y136F mutation was not detected in any of the 53 samples collected in WA nor was it found in samples taken from South Australia (Fig 1). Alarmingly, all of the six assays from New South Wales and the couple run from infected wheat tissue from Tasmania contained high levels of mutant Y136F DNA. Indicating that the Y136F mutation is highly prevalent in the wheat powdery mildew population in the regions sampled in both New South Wales and Tasmania and is by far the dominant genotype in the local area. The entire length of the *Cyp51* gene was sequenced from all samples where the Y136F mutation was detected. The secondary mutation S509T was not detected. This is the first time mutations in the triazole target gene have been found in *Bgt* in Australia. .

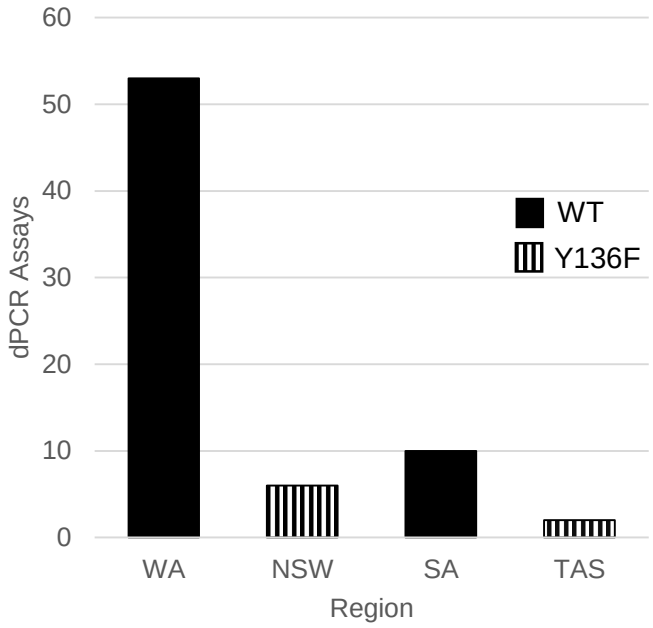

Figure 1. The number Digital PCR assays run on tissue samples collected in the 2015 Australian growing season containing the *Cyp51* Y136F mutation in wheat powdery mildew. Samples have been analysed by region, encompassing wheat tissue collected in WA (Western Australia), NSW (New South Wales), SA (South Australia) and TAS (Tasmania). Mutant Y136F containing samples are stripped and wild-type (WT) samples are given as filled bars.

Conclusion

Wheat powdery mildew was primarily a problem for WA wheat growers during the 2015-2016 season, with infection reported over most of the wheatbelt. Although, not as prevalent in the east of Australia, many wheat growers in the south eastern high rainfall areas reported some degree of infection. Previous mutation screening technologies conducted on wheat samples have failed to detect any mutational changes in the triazole target gene, *Cyp51*. The application of dPCR has allowed a high-throughput, fast turnaround molecular mechanism to screen whole powdery mildew samples containing different mutations, simultaneously. In the case of barley powdery mildew, the Y136F mutation was first detected in 2009 with the more devastating combination of Y136F + S509T found only seasons later, where the resistance afforded by the combination of the two mutations was already clearly having an impact in field control.

Prior to 2015, no *Cyp51* mutations had been detected in any wheat powdery mildew samples screened by conventional PCR. In the first year of dPCR analysis, the precursor or gateway Y136F mutation was detected in *Bgt* populations in Tasmania and New South Wales but as yet not in WA. Thankfully the combination of the Y136F with the S509T mutation has not been detected, however the risk of its development is high if effective management strategies are not implemented to control *Bgt* in seasons to come.

Key words**: Powdery mildew, wheat, fungicide resistance, monitoring, resistance detection**, **fungicide management strategies**

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