Viable solutions for barley powdery mildew

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KEY MESSAGES

- Barley powdery mildew caused by the obligate biotroph *Blumeria (Erysiphe) graminis hordei (*Bgh) is estimated to be the most important disease of barley in Western Australia resulting in ca. $33m in losses (Murray and Brennan, 2010). At present the strategies for management include the use of resistant cultivars and the application of fungicides.
- Bgh exists in numerous races each of which has a specific set of avirulence (*Avr*) genes. Knowledge of the frequency of *Avr* genes in the population can be used to decide which major resistance (*R*) genes should be deployed to control the disease. But as Bgh is notorious for mutating to confer resistance, new methodologies are needed to exploit this information.
- Recessive alleles on the barley *Mlo* gene (*mlo)* confer resistance to almost all known isolates of Bgh. The resistance mediated by *mlo* is independent to that conferred by the *R* genes. Since the 1970s *mlo* resistance alleles have been used extensively in cultivar development (Tacconi et al. 2006). The durability of *mlo* provides an advantage over the often short-lived resistance conditioned by *R* genes. Currently *mlo* continues to provide long-lasting protection against colonisation by all known Bgh pathotypes.
- Fungicides have been used to control Bgh in the absence of *mlo* and effective *R* genes. Bgh is deemed to be of high inherent risk of developing fungicide resistance (Brent and Holloman, 1998). Hence, close monitoring of fungicide effectiveness is central in the management of barley powdery mildew. Surveying field isolates can detect early stages of fungicide resistance before it becomes severe enough to cause practical problems. Several different chemical classes of fungicides are examined for their *in vivo* control of Bgh. Triazoles continue to be effective although we have preliminary indications of reduced efficacy of these and other fungicides.

AIMS

- Perform a pathotype survey to determine which *R* genes could be utilised in the control of WAs Bgh population.
- Develop a robust and high throughput method to screen large numbers of Bgh isolates for putative fungicide resistance—the sentinel test.
- Establish fungicide resistance benchmarks and survey levels of fungicide sensitivity within WA's Bgh population.

METHODS

Isolate collection

The 64 isolates of Bgh used in these studies were collected from barley crops in southern WA. All isolates were purified as single spore isolates on untreated leaf segments (barley cv. Baudin) on water agar containing benzimidazole. Isolates were subcultured at 10 day intervals.

Pathotype survey

Each Bgh isolate was used to inoculate 25 near isogenic barley Pallas lines, with each line carrying either one or two *R* genes at the *Mla* locus. Current WA cultivars Barque and Dash were also included with Baudin, a highly susceptible cultivar used as a positive control. The cultures were incubated and each isolate and line combination was given a disease score according to the level of pustule development at 7 days, with 1 representing complete resistance and 5 complete susceptibility. Lines consistently scoring 3 and above were considered to be defeated.

Fungicide resistance tests

The Sentinel Test

Barley seeds (cv. Baudin) were soaked in calibrated concentrations of fungicides (Azoxystrobin and Fluquinconazole). The seeds were sown at two locations in southern WA into crops with Bgh infections. The number of pustules on plants from both untreated and fungicide treated seeds were counted three weeks after sowing. Isolates from both sets of plants were collected and cultured as above pending *in vivo* testing.

Fungicide Resistance Benchmarks

Three Bgh isolates were randomly selected to infect segments of untreated leaf tissue (cv. Baudin) before being transferred onto benzimidazole agar plates amended with one of seven fungicides. The cultures were incubated and the mean pustule development after 7 days was obtained for each isolate and fungicide combination. The data from three replicates was used to determine the minimum inhibitory concentration (MIC) for each fungicide so that a resistant phenotype could be easily recognised. Fifty-four Bgh isolates were tested at the fungicides' MIC and the scale described previously was used to score the mean pustule development at 7 days post infection. Ratios between the mean pustule development of an isolate on the fungicide plates to that of the control were calculated and used to identify isolates showing fungicide resistance.

RESULTS

Pathotype Survey

We have surveyed 64 Bgh isolates collected from 11 sites in southern WA and so far our results show virulence diversity among the isolates indicating the existence of different Bgh pathotypes in WA (Table 1). The results showed that *mlo* continues to provide a source of resistance along with several other dominant genes. The WA cultivar Barque consistently scored moderately susceptible to susceptible, while Dash was moderately resistant.

Table 1 **Differential responses of Pallas lines to powdery mildew isolates**

Line description	Albany	South Perth	Katanning	Gairdner	Mount Barker	Broomehill	Medina	Esperence
PALLAS Isoline P2 (Ma3)	2	3	3	3	3	2	3	3
PALLAS Isoline P6 (Ma7+MLG2)	2	3	3	2	2	4	3	4
PALLAS Isoline P10 (Ma12)	1	2	3	3	2	1	2	5
PALLAS Isoline P11 (Ma13+mlRu3)	2	2	2	2	1	1	1	3
PALLAS Isoline P13 (Ml402)	3	4	3	3	2	3	3	4
PALLAS Isoline P14 (M41/145)	1	2	1	3	4/3	1	4/3	1
PALLAS Isoline P15 (MRu2)	5	4	3	4	4	3	5	4
PALLAS Isoline P17 (Mk)	4	5	4	5	5	5	2	5
PALLAS Isoline P18 (Mm)	1	4	1	5	3	3	5	4
PALLAS Isoline P22 (mlo5)	1	3	1	1	1	1	1	2
PALLAS Isoline P23 (MLa)	2	5	2	5	4	5	3	5
Baudin	5	5	5	5	5	5	5	5
Barque	3	3	4	3	2	5	4	4
Dash	2	3	1	2	1	2	2	2

Fungicide resistance

Mutations conferring fungicide resistance occur randomly and at a low rate. Therefore screening an appropriate number of isolates to detect resistance is a large task. Through means of the sentinel test, a total of 9088 pustules were counted on the control plants, denoting the effective number of spores screened. The azoxystrobin treated seeds had 3308 and the fluqinconazole 995, providing candidate isolates for fungicide resistance assessment. Isolates from these collections are being tested as we write.

The MIC was estimated for seven fungicides (Table 2) based on the observations from three isolates. Of the triazoles, triadimefon had the lowest MIC of 1 mg/L and the value for epoxiconazole was also very low at 5 mg/L. Tebuconazole, azoxystrobin and thiabendazole all had MIC's of 15 mg/L followed by prochloraz which only took affect at 100 mg/L. Chlorothalonil was omitted from further testing because of its very high MIC. When screened on the calibrated fungicide MIC's, several isolates showed decreased responsiveness to the fungicides. Currently one isolate has putative resistance to azoxystrobin, another to prochloraz and five more isolates showed low sensitivity to the triazoles tebuconazole, triadimefon and epoxiconazole. Further testing into the level of fungicide resistance of these isolates is being undertaken as we write.

Table 2 **Chemical class and the minimum inhibitory concentration (MIC) of fungicides used**

Fungicide name	Chemical class	MIC (mg/L)
Epoxiconazole	Triazole	5
Triadimefon	Triazole	1
Thiabendazole	Benzimidazole	15
Chlorothalonil	Chloronitrile	> 500
Tebuconazole	Triazole	15
Prochloraz	Imidazole	100
Azoxystrobin	Strobilurin	15

CONCLUSION

Powdery mildew is a widespread disease on Western Australian barley crops. It has been demonstrated that *mlo* remains fully effective against all Bgh isolates surveyed. It would therefore seem useful for all barley cultivars to carry *mlo;* however breeders resist its incorporation into cultivars as it is linked to yield penalties. A second means of protecting against Bgh colonisation is to include one or more undefeated *R* genes. This strategy requires extensive and continual field surveys of Bgh *Avr* genes in order to deploy cultivars with appropriate *R* genes. A new strategy based on minor gene resistances may therefore be the most promising route (Yu et al. 2001).

As the trend for more frequent fungicide application continues, the evolutionary potential of the pathogen population increases, where those individuals with reduced sensitivity to the fungicide may have an advantage. It was found in this experiment that the Western Australian Bgh population was on the whole highly responsive to the triazole, strobulurin and benzimidazole classes of fungicides but 7 isolates displayed reduced sensitivity. The application rate of prochloraz and chlorothalonil would possibly be considered too high for practical purposes.

For the effective protection of barley from powdery mildew disease, an integrated approach including the use of resistant cultivars and fungicide application is required. Constant monitoring of the changes in the population is essential for ensuring longevity of control.

KEY WORDS

Blumeria graminis hordei, fungicide resistance, genetic resistance

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