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BARLEY NET BLOTCH: SURVEY OF SENSITIVITY OF *PYRENOPHORA TERES* TO FUNGICIDES

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by

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ABSTRACT

Barley net blotch first came to prominence in the late 1970s, and was the major disease in all winter barley growing areas. Control was achieved with the newly introduced DMI fungicides, propiconazole and prochloraz, and in recent years the disease has been at more moderate levels. The main fungicides used to control net blotch continued to be those in the DMI group, and pathogen sensitivity has been monitored since the 1980s.

In this project, net blotch samples were taken from 25 winter barley crops in England during the early summer of 1999. Over 200 isolates of the pathogen, *Pyrenophora teres*, were obtained from 22 of these crops and tested for sensitivity to four DMI fungicides (propiconazole, flusilazole, epoxiconazole and prochloraz), in a repeat of a similar survey in 1996. In all cases, the mean sensitivity of the isolates was similar to those found three years previously. Therefore, the marked shift in sensitivity that had been occurring from 1988 to 1996 had stopped. The close relationship in sensitivity of isolates between propiconazole and flusilazole was apparent in 1999, as it had been in 1996.

For the first time, isolates were screened to see if any resistance to the strobilurin fungicide azoxystrobin was present in the UK *P. teres* population. A total of 176 isolates were screened and no resistance was detected.

The anilinopyrimidine fungicide, cyprodinil offers another alternative to the DMIs for net blotch control. Sensitivity to this fungicide was also examined for the first time in 1999. In general, isolates of *P. teres* were very sensitive to cyprodinil, but the finding of three isolates that made some growth in the presence of the fungicide indicates a variability in sensitivity levels in the population. If farmer use of cyprodinil increases, a shift in the sensitivity of the population might be expected in the future.

The results of the survey have been of value in formulating advice to farmers on net blotch control, and were used in the production of the FRAG-UK/HGCA leaflet, 'Guidelines for preventing and managing fungicide resistance in cereal pathogens'.

SUMMARY

Objectives

The objectives of this project were:-

(a) To determine the sensitivity of *P. teres* to six fungicides with activity against the pathogen.
(b) To measure any shift in sensitivity to four of the fungicides which were screened in 1996 or earlier.
(c) To measure for the first time the sensitivity of the pathogen to two newly introduced fungicides.
(d) To provide advice to farmers on the best strategy for the control of net blotch, based on the results of the project.

Introduction

Net blotch of barley, caused by *Pyrenophora teres*, can cause heavy losses in yield if not adequately controlled. The main group of fungicides used against net blotch are the DMIs. The sensitivity of isolates of the net blotch pathogen has been checked since 1989, most recently in 1996 (HGCA Project Report No.132) and a continuing decline in sensitivity to propiconazole and prochloraz was found. In 1996 two of the more recently developed DMIs, epoxiconazole and flusilazole, were tested for the first time. Control of net blotch is still largely dependent upon DMI fungicides but other chemistry has become available to farmers, in the form of azoxystrobin and cyprodinil (strobilurin and anilinopyrimidine fungicides respectively).

In 1998 the disease was widespread and some difficulties in control were reported from south-west England. The summer of 1999 was therefore an opportune time to monitor the sensitivity of *P. teres* again, to a range of fungicides, specifically:
(a) propiconazole and prochloraz to check if sensitivity is still declining,
(b) epoxiconazole and flusilazole to detect any shift in sensitivity since 1996 and
(c) azoxystrobin and cyprodinil to gather information on sensitivity before the use of these fungicides becomes more widespread.

The information gained will be of use to farmers in planning strategies for the control of net blotch, whilst minimising the risk of fungicide resistance

Materials and Methods

Sampling of crops

Samples were collected in mid-June 1999 from randomly selected infected crops. In total, 25 crops were sampled and the leaf material was sent to the ADAS Plant Diagnostic Laboratory at Wolverhampton for pathogen isolation and fungicide screening.

Isolation of *P. teres*

Approximately 20 infected leaves were chosen from each field sample for culturing of *P. teres* and a final selection was made of 10 clean cultures per site for sensitivity testing. The initial isolations from the leaf material were made on to PDA.

Sensitivity testing

For testing the DMI fungicides, isolates were sub-cultured on to PDA that was either unamended or contained propiconazole, epoxiconazole, flusilazole or prochloraz at one of four concentrations (0.2,1.0, 5.0 or 25 μg/ml). The percentage growth rate of the cultures on plates containing fungicide compared to that on unamended agar was calculated and ED_{50} values (the fungicide dose required to reduce *P. teres* growth on agar plates by 50%) were determined for each isolate on each DMI fungicide. Measurements were made after 5 days growth at 18°C in the dark.

A similar initial procedure was carried out using cyprodinil. A selection of 40 isolates exhibiting the highest apparent ED_{50} values were then screened by a specific method for that fungicide, based on methodology supplied by Novartis (C. Mills, pers. comm.). This involved the sub-culturing of isolates by the transfer of hyphal tips on to the fungicide-containing media. These 40 isolates were thus re-tested at the same range of concentrations described above.

For azoxystrobin, a spore germination assay was employed, following a procedure supplied by Zeneca (S. Heaney, pers.comm.). After initial preparatory stages, agar plates seeded with spores were incubated in darkness at 6°C for 24h. In this case a single discriminatory dose of 10 μg/ml was used, with any isolates having spores germinating at that level of fungicide being considered resistant. This method was used because resistance to strobilurin fungicides occurs as a 'single step', in a similar way to benzimidazole resistance, rather than as a series of small shifts that occurs, for example, with DMI fungicides.

Results

DMI fungicides

The mean ED_{50} values for *P.teres* isolates are presented in Table 1. These are compared to the results reported by Hims (1993) and Locke (1996) in Tables 2 and 3.

Table 1. ED_{50} values for isolates of *P. teres*, 1999

Fungicide	No. of isolates	Mean ED_{50} (µg/ml)	Standard deviation
Propiconazole	215	0.686	± 0.405
Flusilazole	219	0.338	± 0.287
Epoxiconazole	219	0.158	± 0.143
Prochloraz	216	0.147	± 0.121

The highest ED_{50} values found for propiconazole and flusilazole were 5.0 and 5.6 respectively, and those for epoxiconazole and prochloraz were 1.3 and 1.5 respectively. These maximum levels are similar to those found in 1996.

Table 2. ED_{50} values for propiconazole and prochloraz, 1988 - 1999

Fungicide	Year	Mean ED_{50} (µg/ml)
Propiconazole	1988	0.087
	1991	0.15 - 0.30*
	1996	0.766
	1999	0.686
Prochloraz	1988	0.0032
	1991	0.06 - 0.14*
	1996	0.221
	1999	0.147

* estimates based on % growth rates on 0.5 and 1.0 µg/ml propiconazole and 0.1 and 1.0 µg/ml prochloraz recorded after 7 and 14 days growth.

Table 3. ED_{50} values for flusizazole and epoxiconazole, 1996 - 1999

Fungicide	Year	Mean ED_{50} (µg/ml)
Flusilazole	1996	0.551
	1999	0.338
Epoxiconazole	1996	0.233
	1999	0.158

Full data sets for all four DMI fungicides were obtained for 212 isolates and correlation coefficients were determined between each pair of fungicides to establish how closely related these were in terms of sensitivity. This is of importance, as farmers wish to apply two DMI fungicides during the season, and should try to avoid closely related produced to minimise resistance risks. The results are given in Table 4, together with those reported by Locke in 1996, based on 250 sets of data.

Table 4. Sensitivity relationship of DMI fungicides

Fungicides	Correlation coefficient 1999	Correlation coefficient 1996
Propiconazole:flusilazole	0.784	0.740
Propiconazole:epoxiconazole	0.230	0.475
Propiconazole:prochloraz	0.318	0.496
Flusilazole: epoxiconazole	0.386	0.467
Flusilazole: prochloraz	0.306	0.342
Epoxiconazole:prochloraz	0.061	0.496

The results confirm that the closest relationship within the group of four DMIs examined, in terms of sensitivity, is between propiconazole and flusilazole.

In the earlier net blotch study, in 1996, it was possible to examine the interaction of *P. teres* sensitivity and previous cropping in the sampled fields and also between sensitivity and number of DMI sprays applied to the crops. In 1999, the data set did not make such an examination meaningful (e.g. in 1999, 2 crops had no DMI fungicides applied, 16 crops had 1 DMI spray and 4 crops had 2 DMI sprays). However, as the sensitivity of the DMI fungicides has changed very little from 1996 to 1999, the data from these two survey years could be combined. The results of this combined analysis are presented in Tables 5 and 6.

Table 5. Effect of number of DMI sprays on fungicide sensitivity, 1996 and 1999

		Mean ED_{50} value (μg/ml)			
No. of DMI sprays applied	No. of crops	Propiconazole	Flusilazole	Epoxiconazole	Prochloraz
2 or 3	13	0.80	0.65	0.24	0.20
1	30	0.74	0.43	0.20	0.19
0	5	0.61	0.23	0.10	0.10

The results show a clear trend, with all four DMI fungicides, towards decreasing sensitivity with increasing DMI usage in the crop.

Table 6. Effect of previous cropping on DMI sensitivity, 1996 and 1999

		Mean ED_{50} value (μg/ml)			
Previous crop	No. of crops	Propiconazole	Flusilazole	Epoxiconazole	Prochloraz
Barley	19	0.87	0.59	0.24	0.22
Non-barley*	29	0.65	0.40	0.19	0.17

* 24 winter wheat, 2 linseed, 1 each of potato, grass and peas.

The *P. teres* populations were generally less sensitive to DMI fungicides in those fields where barley had been grown the previous year. This would suggest that a selection pressure had already taken place in the pathogen population the previous season, as DMI usage would probably have occurred in that crop. Therefore, a pre-selected population of decreased sensitivity, would be present on trash and volunteers ready to infect the current season crop.

Azoxystrobin

A total of 176 isolates of *P. teres* were induced to produce spores, enabling testing to take place. No sporing isolates were obtained from three crop samples, and the number of isolates screened from the remaining 22 crops ranged from 2 to 10 (mean of 8 per crop). The spores of none of the isolates tested germinated on agar amended with 10 μg/ml azoxystrobin. Therefore no resistance to that fungicide was detected in the 1999 survey.

Cyprodinil

Of the 40 least sensitive isolates from the first screen, that were taken to the more detailed second screen, only 3 made growth at 0.2 μg/ml cyprodinil. The MIC values recorded for these were 1.0, 5.0 and 25.0 μg/ml, with estimated ED_{50} values of >0.1, 0.6, and 2.0 μg/ml respectively. These isolates originated from three separate crops, none of which had received a cyprodinil application.

Discussion

The results of the 1999 survey show that the decline in sensitivity to DMI fungicides found in the *P. teres* population during the period 1988 to 1996 has halted. The mean sensitivity of the isolates the each of the four DMIs has marginally increased in the three seasons between 1996 and 1999. The close relationship between propiconazole and flusilazole, in terms of individual isolate sensitivity, that was reported in 1996, has been confirmed. Only one of these two active ingredients should be used in a net blotch control programme, and if another treatment is necessary a different DMI or a fungicide from another chemical group should be used. In this way any resistance risk to the DMIs can be minimised, and the likelihood of effective control increased.

For this first time in this series of HGCA-funded projects, isolates of *P. teres* were checked for resistance to azoxystrobin. Unlike the DMIs, where gradual shifts in sensitivity are detected, with azoxystrobin a single large shift to a fully resistant strain can be expected. Isolates were therefore tested at the recommended discriminatory dose of 10 μg/ml to detect spores germinating at that concentration. In this survey no such resistance was detected, but in view of the problems encountered with the strobilurin fungicides and wheat mildew, further surveillance is advisable in future seasons.

The third type of fungicide screened, again for the first time in 1999, was cyprodinil. This had only been applied to one of the survey crops, and has not made significant market penetration, so far. *P. teres* sensitivity to cyprodinil appears to be very high at present, but the detection of 3 isolates that made some growth on the amended agar indicates that there is some variation in sensitivity in the field population. If this product becomes more widely used in barley crops, a shift in sensitivity might be expected. At present the fungicide can make a useful contribution to a resistance strategy, in net blotch control programmes.

The project has been valuable in establishing the present status of *P. teres* sensitivity to three types of fungicide, DMIs, strobilurins and anilinopyrimidines. The findings on DMIs can be related to those reported in earlier studies, so giving the farming industry an insight into a dynamic situation. A further examination of the *P. teres* population in the near future, (2001 or 2002) would add to the information gathered on all three types of fungicide, to enable the industry to keep up to date on the resistance situation with net blotch in barley. Meanwhile, alternation of fungicide resistance groups is recommended, and FRAG-UK guidelines, published in 2000, should be followed, to protect the widely used DMI and strobilurin fungicides. The results presented here for DMI fungicides were used in the preparation of the Guidelines, but those for cyprodinil and azoxystrobin were obtained after the publication date. They will be of value in the next revision of the Guidelines.

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