HGCA

PROJECT REPORT No. 132

BARLEY NET BLOTCH: SURVEY OF SENSITIVITY TO DMI FUNGICIDES

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by

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Abstract

In a survey of 30 winter barley crops during 1996 isolates of *Pyrenophora teres* were found to be less sensitive to propiconazole and prochloraz than in 1991. A close correlation was found between the sensitivity of isolates to propiconazole and flusilazole. Positive correlations were also found between all four demethylation inhibitory (DMI) fungicides tested, propiconazole, prochloraz, expoxiconazole and flusilazole.

Pathogen sensitivity was lower following the use of two or three DMI sprays in the sampled crop, rather than where one such spray had been used. Lower sensitivity was also found in crops which followed barley rather than another type of crop. In fifteen crops net blotch levels were recorded at growth stage 71 - 73 and reduced sensitivity occurred in those crops with the lowest disease levels. There was no significant difference in sensitivity levels between samples from different parts of England and from Scotland.

Objective

The objective of this project was to determine the level of sensitivity of populations of *P. teres* to a range of DMI fungicides. A previous study conducted for the HGCA took place in 1991 (Hims, 1993). Any shifts in the population for sensitivity to propiconazole and prochloraz since 1991 would be revealed by the 1996 survey. By also screening for sensitivity to two newer DMI fungicides, expoxiconazole and flusilazole, an understanding could be achieved of how closely related the four fungicides are in terms of cross-resistance.

Introduction

In France, efficacy of some DMI fungicides for the control of barley net blotch has declined in the past few years. Fungicide resistance is suspected to be the reason for the control failures. There have been no confirmed cases of disease control failure in the UK but, in 1995, samples of *P. teres* from a trial in Devon were found to have decreased sensitivity to propiconazole. As control of net blotch is totally reliant upon DMI fungicides (flusilazole, epoxiconazole, propiconazole and prochloraz mainly) any widespread decrease in sensitivity would have very serious implications for barley growers. The disease can develop rapidly in wet warm weather and in early 1996 infection was common in barley crops. It was therefore opportune to collect material in the spring for sensitivity screening. A dry early spring delayed the epidemic but by June many crops were affected and samples were easily obtained.

Materials and Methods

Sampling

Samples were collected in May and June from randomly selected infected crops, 50% of which were being used as part of the CSL/ADAS National Survey of Winter Barley Diseases. Leaf material from approximately 40 crops was sent to the ADAS Plant Clinic at Wolverhampton.

Isolation

Plants from a sub-sample of thirty crops were selected for pathogen isolation and infected leaf material was plated on to PDA. About 20 leaves were chosen from each sample for culturing of *P. teres* and a final selection was made of 10 clean cultures per site for sensitivity testing.

Sensitivity Testing

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, 25.0 µg/ml). The agar plates were incubated at 15°C for three days. Colony growth rate from the inoculation source was measured and that on the fungicide amended agar was compared to that on the unamended agar.

ED_{50} determination of the cultures

The percentage growth rate of the cultures compared to that on unamended agar was calculated and ED_{50} values were determined for each isolate on each fungicide.

Results

The mean ED_{50} values for *P. teres* isolates are presented in table 1. Hims (1993) gave ED_{50} values for isolates in 1988 and growth measurements in 1991 for propiconazole and prochloraz only. These data are compared with the 1996 results in table 2 and show a continuing decline in sensitivity.

Table 1. ED_{50} values of isolates of *Pyrenophora teres,* 1996

Fungicide	No. of Isolates	Mean ED_{50} (µg/ml)	Standard Deviation
Propiconazole	259	0.766	± 0.866
Flusilazole	259	0.551	± 1.076
Epoxiconazole	262	0.233	± 0.382
Prochloraz	263	0.221	± 0.411

Table 2. ED_{50} values 1988 - 1996

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

* estimates based on % growth rates on 0.5 and 5.0 µg/ml propiconazole and 0.1 and 1.0 µg/ml prochloraz recorded after 7 and 14 days growth.

Hims presented his 1992 survey data in terms of growth rate of the pathogen on amended agar in comparison to unamended agar. He did not calculate individual isolate ED_{50} values as the tests were done on only two concentrations of fungicide rather than the four in this survey. The percentage of isolates in each growth rate category in 1991 and 1996 for propiconazole @ 5.0 µg/ml and prochloraz @ 1.0 µg/ml are presented in table 3.

Table 3. Growth rate of isolates on fungicide on amended agar compared to unamended agar, 1991 and 1996.

% growth rate on amended agar	% of isolates			
	Propiconazole (5.0 µg/ml)		Prochloraz (1.0 µg/ml)	
	1991	1996	1991	1996
0 -5	22	27.3	7	13.6
6 - 10	9	9.1	18	4.5
11 - 15	15	9.1	21	11.3
16 - 20	13	10.6	22	15.5
21 - 25	16	12.5	12	14.7
26 - 30	10	7.6	11	11.7
31 - 35	5	9.1	2	8.0
36 - 40	3	5.7	4	9.1
41 - 45	2	3.4	1	4.5
46 - 50	2	3.0	1	2.6
51 - 55	1	0.8	0	1.1
56 - 60	1	1.1	0	1.5
61 - 65	1	0.4	1	0.4
66 - 70	0	0.0	0	0.8
71 - 75	0	0.0	0	0.0
76 - 80	0	0.4	0	0.4
81 - 85	0	0.0	0	0.4

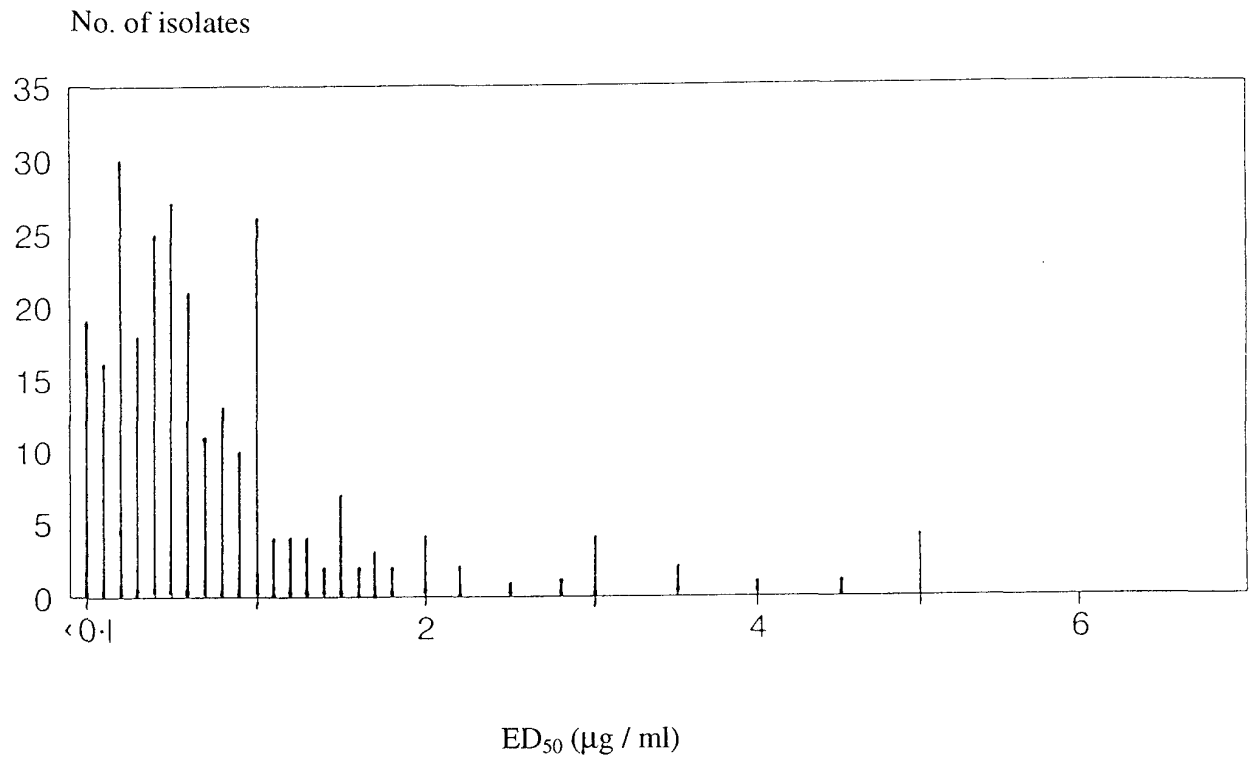

Figure 1. Distribution of ED_{50}s for propiconazole

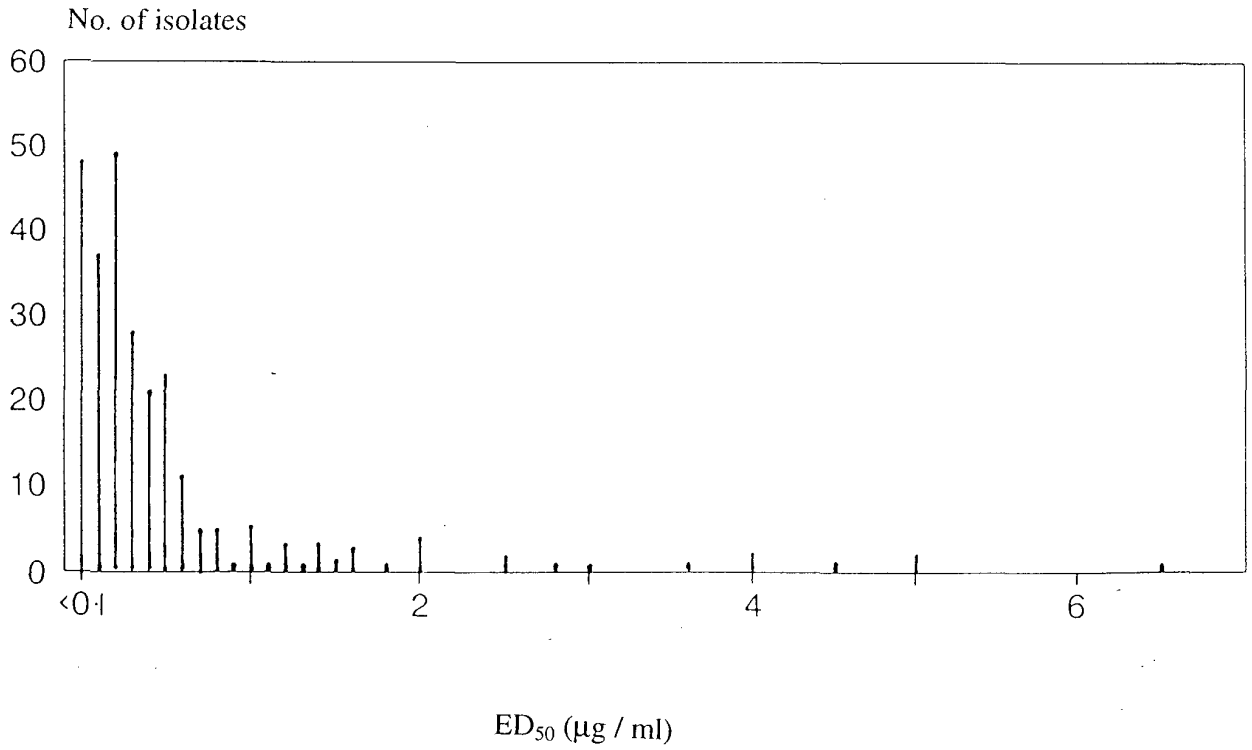

Figure 2. Distribution of ED_{50}s for flusilazole

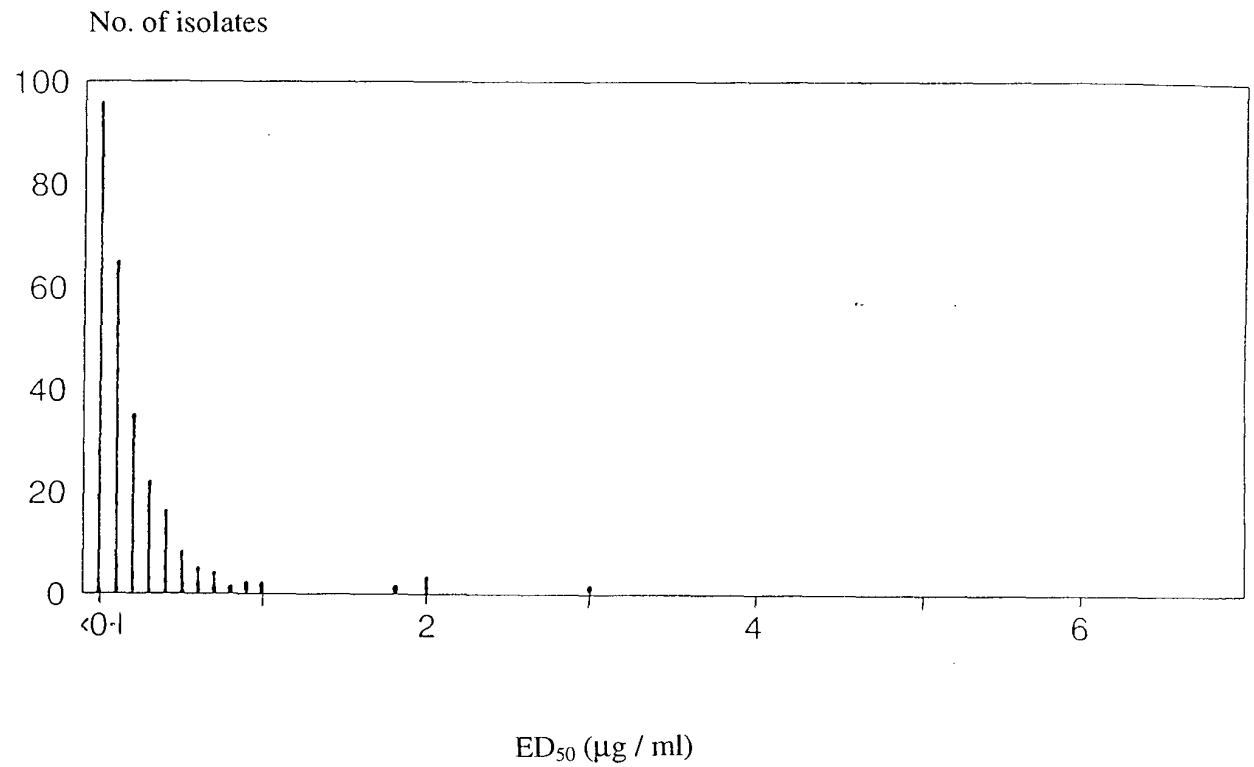

Figure 3. Distribution of ED_{50}s for epoxiconazole

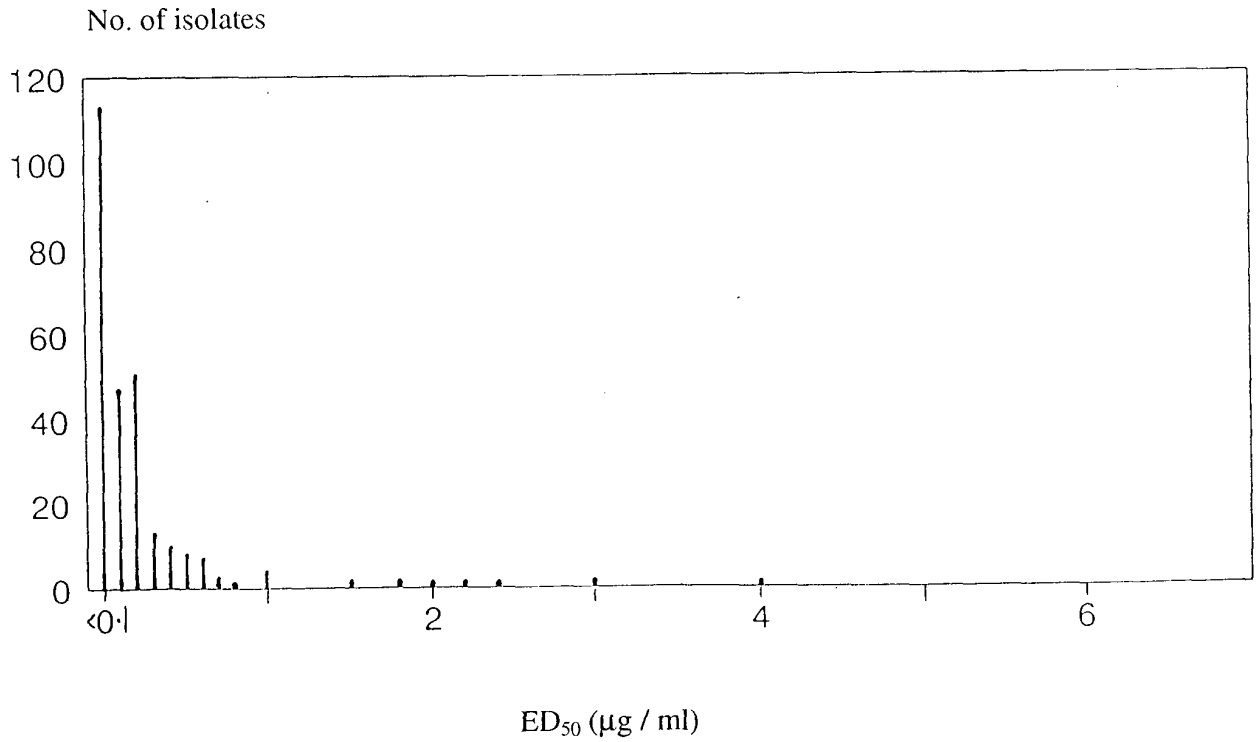

Figure 4. Distribution of ED_{50}s for prochloraz

The distributions of isolates of different ED_{50} values are shown in figures 1 - 4 for the four fungicides. There is no clear bimodal distribution to indicate two distinct pathogen sub-populations. There are a few isolates at one end of the continuum with notably higher ED_{50} values. The number of isolates with ED_{50} values greater than the population mean plus two standard deviations is 13 for propiconazole (≥ 2.6 µg/ml), 10 for flusilazole (≥ 2.8 µg/ml), 5 for epoxiconazole (≥ 1.1 µg/ml) and 7 for prochloraz (≥ 1.1 µg/ml). These represent 4.9%, 3.8%, 1.9% and 2.7% of the populations respectively.

Full sets of ED_{50} data were obtained from 250 isolates and correlation coefficients were determined between each pair of fungicides to establish how closely related these were in terms of sensitivity. The results are given in table 4 and show that propiconazole and flusilazole are considerably closer than any other pairing.

Table 4. Relationship of fungicides

Fungicides	Correlation coefficient
Propiconazole : Flusilazole	0.740
Propiconazole : Epoxiconazole	0.475
Propiconazole : Prochloraz	0.496
Flusilazole : Epoxiconazole	0.467
Flusilazole : Prochloraz	0.342
Epoxiconazole : Prochloraz	0.496

There was an indication that higher ED_{50} values were obtained with slower growing isolates. This was confirmed by correlation coefficients obtained from these 250 isolates but the relationship was not particularly strong: propiconazole -0.227; flusilazole -0.165; epoxiconazole -0.187; prochloraz -0.343.

Relationships between mean ED_{50} values and geographic location, DMI fungicide use in the crop, and previous crop were examined. Fifteen of the samples came from crops in the winter barley disease survey so details of net blotch infection levels were known at growth stage 71-73. These factors are presented in tables 5-8.

Table 5. ED_{50} values in crops from various geographic locations.

Location	No. of crops	Mean ED_{50} (µg/ml)			
		Propiconazole	Flusilazole	Epoxiconazole	Prochloraz
Scotland	3	0.56	0.23	0.11	0.17
North of England	7	0.67	0.58	0.24	0.13
East/S.E. England	10	0.68	0.73	0.25	0.24
W. Midlands / S.W. England	10	0.75	0.44	0.21	0.26

Table 6. Effect of previous cropping on ED_{50} values

Previous crop	No. of crops	Mean ED_{50} (µg/ml)			
		Propiconazole	Flusilazole	Epoxiconazole	Prochloraz
Barley	12	0.95	0.75	0.30	0.27
All other crops*	15	0.64	0.40	0.18	0.17

* 12 wheat; 2 linseed; 1 potato.

Table 7. Effect of DMI fungicide sprays on ED_{50} values.

No. of DMI sprays	No. of crops	Mean ED_{50} value (µg/ml)			
		Propiconazole	Flusilazole	Epoxiconazole	Prochloraz
2 or 3	9	0.86	0.80	0.27	0.21
1	15	0.72	0.48	0.24	0.24
0	3	0.73	0.30	0.10	0.10

Table 8. Net blotch incidence and ED_{50} values.

Mean net blotch infection level* (% leaf area)		No. of crops	Mean ED_{50} value (µg/ml)			
Leaf 1	Leaf 2		Propiconazole	Flusilazole	Epoxiconazole	Prochloraz
2.55	14.49	7	0.64	0.42	0.16	0.16
0.27	0.99	8	1.00	0.94	0.35	0.23

* Higher infection level group : range leaf 1 0.92 - 7.96, leaf 2 7.52 - 29.96
Lower infection level group : range leaf 1 0 - 0.84, leaf 2 0 - 1.88

These results indicate that there is no significant variation in mean ED_{50} levels across England and Scotland. Where the sampled crop followed barley in 1995 higher ED_{50} values were encountered suggesting that a selection pressure had already taken place in the pathogen population the previous year. There was a trend towards higher ED_{50} values following more intensive use of DMI fungicides in the sampled crop but this effect was not as marked as that given by the previous cropping. The sample was too small to allow an examination of the interaction of these two factors.

The relationship of ED_{50} levels and disease levels was interesting in that crops with greater net blotch infection had lower ED_{50} values for the four fungicides. It is possible that this result indicates that where control had been good, and lower disease levels were present at the end of the season, the residual *P. teres* population was less sensitive to the fungicides.

Discussion

The results of the 1996 survey indicate that the *P. teres* population is continuing to decrease in sensitivity to propiconazole and prochloraz. As there is a very high positive correlation between propiconazole and flusilazole, and weaker positive correlations between other pairs of DMI fungicide examined, this would suggest that there is a general shift towards decreasing sensitivity to the DMI fungicide group as a whole. There was no clear bimodal distribution of the population in terms of ED_{50} values of individual isolates but a small percentage of them (2% - 5% depending upon the fungicide) were beyond two standard deviations from the mean towards the more resistant end of the continuum.

The study has shown that decreased sensitivity is associated with increased frequency of use of DMI fungicides and with consecutive barley cropping. In the latter case this is probably connected with exposure of the *P. teres* population to two year's use of DMI fungicides in the pathogen life cycle, although information on product use in the preceding crop was not obtained. The DMI products used on the sample crops included propiconazole, flusilazole, epoxiconazole, cyproconazole, tebuconazole and prochloraz but there was no clear relationship between fungicide type and ED_{50} value.

An effect of decreasing sensitivity on net blotch incidence was found. Where the use of DMI fungicides had given good control of the disease the population was found to be less sensitive. This was presumably due to the control of the more sensitive pathotypes in the population during the season, leaving the less sensitive isolates to be sampled. Conversely, where disease levels were higher the population spectrum was greater and ED_{50} values were on average lower. It would be of interest to determine the ED_{50} values of the *P. teres* population in fields in continuous barley production which were subjected to DMI fungicide use each year. Under these circumstances it might be expected that the shift towards decreasing sensitivity would eventually lead to loss of satisfactory disease control.

Control of net blotch is heavily dependent upon DMI fungicides and if treatment for this disease continues to be reliant on these materials a gradual loss of control may be expected. New groups of fungicides will be available soon for use on cereals and some of these control net blotch (e.g. cyprodinil). They should be incorporated into the barley spray programme where net blotch is a problem.

Reference

Hims (1993). Monitoring sensitivity to the DMI group of fungicides in net blotch (*Pyrenophora teres*) of winter barley. Project Report No. 80, Home-Grown cereals Authority, London.