FENPROPIMORPH SENSITIVITY IN *ERYSIPHE GRAMINIS* F.SP. *TRITICI*; SURVEY OF NORTHERN FRANCE 1991-1993.

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ABSTRACT

As part of an annual survey of fungicide sensitivity in cereal powdery mildew, 'single-spore' isolates of *Erysiphe graminis* f.sp. *tritici* were obtained from Northern France in 1991, 1992 and 1993, using a car-mounted Jet Spore Trap. A standard technique was used to measure the sensitivity of all the isolates to the morpholine fungicide fenpropimorph. In 1993, approximately 90% of the isolates were found to be less sensitive to fenpropimorph than wild type sensitive isolates, compared with 55% in 1991. Forty-five *E.graminis* f.sp. *tritici* isolates from various sources were tested against fenpropimorph, tridemorph and fenpropidin, to investigate cross-sensitivity relationships between these three fungicides. While there appeared to be no differences in the responses of the isolates to tridemorph, there was evidence to suggest a correlation between sensitivities to fenpropidin and fenpropimorph.

INTRODUCTION

Annual surveys of fungicide sensitivity in wheat powdery mildew in Northern France were carried out by ICI Agrochemicals (now ZENECA Agrochemicals) between 1991 and 1993. Single spore isolates of *Erysiphe graminis* f.sp. *tritici* were collected using mobile 'Jet Spore Traps'. A standard technique was then used at Jealott's Hill to test samples of the isolates for sensitivity to the morpholine fungicide, fenpropimorph.

Experiments were also carried out on a selection of 45 *E. graminis* f.sp. *tritici* isolates, to observe any correlation between their responses to fenpropimorph, fenpropidin (a piperidine) and tridemorph (a morpholine).

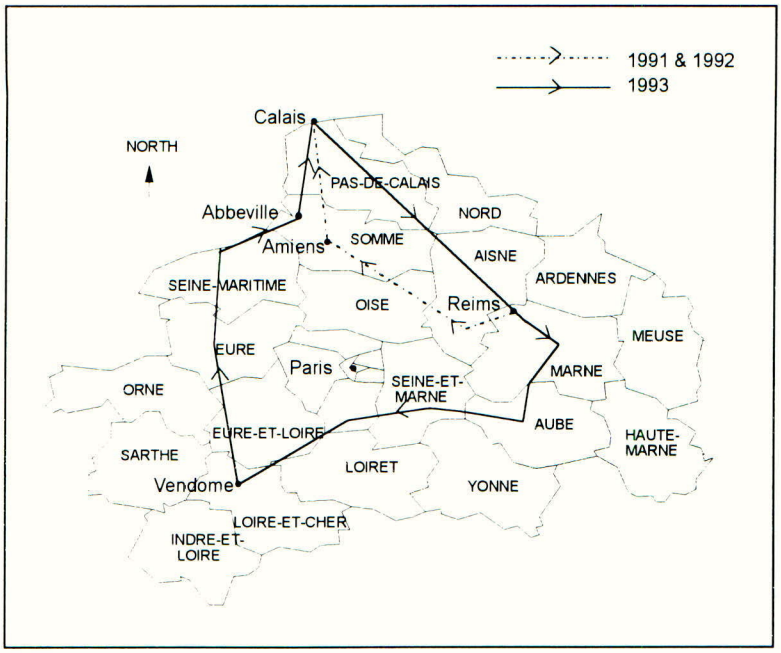
MATERIALS AND METHODS

Collection of 'single spore' isolates

Spores of *E. graminis* f.sp. *tritici* were collected from Northern France in early May (1992 and 1993) or June (1991), using car-mounted Jet Spore Traps (Burkard Manufacturing Co. Ltd., Rickmansworth, UK).

In 1991, 20 'single spore' isolates were obtained from a region between Calais, Reims and Amiens (Figure 1). In 1992, 106 isolates were collected from a similar area. In 1993, the survey area was extended, and 203 isolates were obtained from 9 different *départments* of Northern France, along a route of approximately 800 miles. Isolates from the spore trap were subcultured on untreated wheat prophylls until sufficient inoculum was produced to assay sensitivity to fenpropimorph. Most isolates produced sufficient inoculum within three generations.

Figure 1. Map of Northern France showing spore trap routes in 1991, 1992 and 1993.



Fenpropimorph sensitivity tests

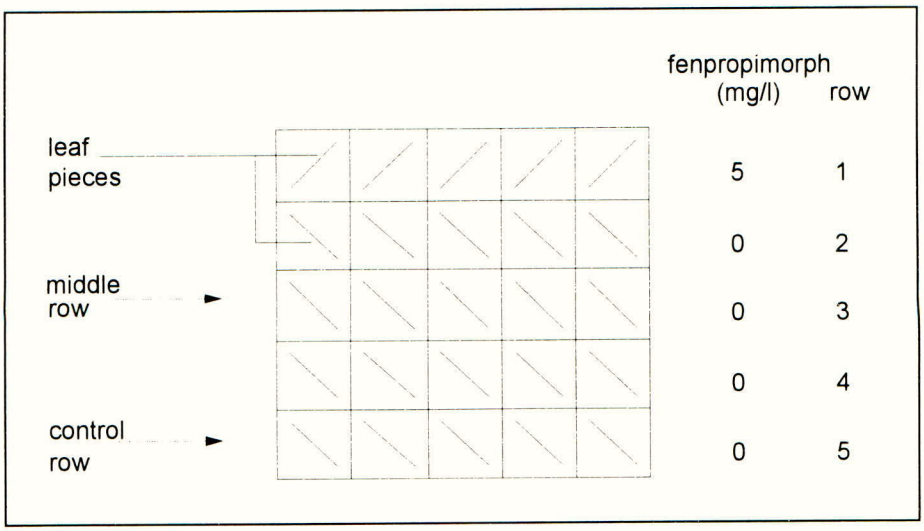
Test design

The test is designed to exploit the vapour active property of fenpropimorph. Vapour from the fenpropimorph solution in the first row of the test dish diffuses across the dish, inhibiting development of sporulating disease on the leaf pieces at a distance from the vapour source dependent on the fenpropimorph sensitivity of the test isolate. Previous work has shown that the powdery mildew on the leaf pieces in the row of wells furthest from the treated row is unaffected by the fenpropimorph vapour, and therefore acts as an untreated control (figure 2). The test enables the fenpropimorph sensitivities of mildew isolates to be qualitatively compared with one another. Quantitative data, such as estimates of LC50 values or resistance factors are not obtained.

Preparation of test dishes

$2cm^3$ non-sterile de-ionised water was placed in 20 wells of a 25 well plastic test dish. Untreated prophyll pieces (approximately 2.5cm long), were cut from 9 day old wheat seedlings (cv. 'Rapier') grown under constant conditions; (DAY [16h]; 21°C, 60% r.h., 8000 lux: NIGHT, 18°C, 95% r.h.). Prophyll pieces were placed diagonally on the surface of the water in the wells, adaxial surface uppermost. $2cm^3$ aliquots of 5mg/l fenpropimorph solution (made up from 'Mistral'; 75% EC) were added to each well of the remaining row of the dish, immediately prior to inoculation. Leaf pieces, as above, were placed on the surface of this solution.

Figure 2. Fenpropimorph sensitivity test in 25 well plastic dish.



Inoculation of test dishes

Test dishes were inoculated using prophyll pieces (10 per dish) that had themselves been inoculated with spores of the test isolate 7 days previously, and subsequently incubated in a CT (constant temperature) room (DAY [16h]; 21°C, 70% r.h., 8000 lux: NIGHT; 18°C, 95% r.h.). Prepared test dishes were inoculated individually in an extracted fume cupboard, using a small wooden settling tower. Test dishes were sealed with non-porous tape after inoculation. Sealed dishes were immediately placed in the CT room, and incubated for 7 days. Two reference isolates, of known fenpropimorph sensitivity, were included in each batch of isolates tested, to detect any between-test variation.

Assessment of the test and data analysis

After 7 days' incubation, the percentage surface area of each leaf piece covered in sporulating disease was estimated and recorded. An illuminated magnifying glass was used to assist assessment. Percentage values on the middle row (row 3), and on the control row (row 5), were totalled separately. The amount of disease on the middle row was then expressed as a percentage of that on the control row.

This value was used to classify isolates into 3 categories, as follows;-

Category	% of control row covered with sporulating disease
LEAST SENSITIVE	>50
INTERMEDIATE	15-50
SENSITIVE	<15

The results are presented in figure 3.

Cross-sensitivity tests

The sensitivity test technique described above was used to investigate the responses

of 45 isolates from various sources to fenpropimorph, fenpropidin and tridemorph. All tests were prepared as described previously, except that either fenpropidin (10mg/l, prepared from Patrol 75% EC), or tridemorph (5mg/l, prepared from Calixin 75% EC) were used instead of fenpropimorph.

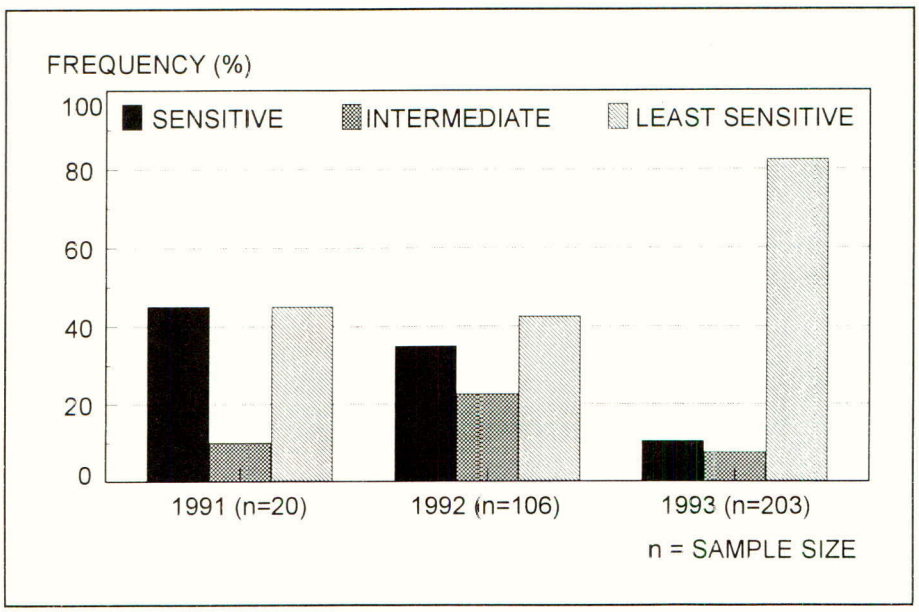
Cross-sensitivity tests were inoculated as described previously. The size of the inoculating tower enabled 3 test dishes, treated with either fenpropimorph, fenpropidin or tridemorph to be inoculated simultaneously with a single isolate. Dishes were sealed with non-porous tape after inoculation, and incubated for 7 days in the CT room. The percentage of the surface area of each leaf piece covered with sporulating disease was then estimated by eye and recorded. On the basis of their responses, the isolates were classified as 'least sensitive', 'intermediate' or 'sensitive' to the 3 chemicals. Any correlations between the responses of the 45 isolates to the three chemicals were noted. The results are displayed in figure 4.

RESULTS

Fenpropimorph sensitivity

Figure 3 reveals that the frequency of isolates classified as 'least sensitive' to fenpropimorph increased from approximately 40% in 1991 to approximately 80% in 1993. This is accompanied by a corresponding decrease in the frequency of 'sensitive' and 'intermediate' isolates, suggesting there has been a decrease in the sensitivity of the wheat powdery mildew population in Northern France as a whole. There appeared to be no important regional differences in fenpropimorph sensitivity within the area sampled.

Figure 3. Fenpropimorph sensitivity in Northern France, 1991-1993.



Cross-sensitivity tests

No differences were detected between the responses of any of the 45 isolates to tridemorph. In all tridemorph tests, disease developed to approximately 100% coverage on all leaf pieces on the fourth and fifth rows of the test dish, and none of the 45 isolates produced disease on the third row. Amending tridemorph rates to 25, 10, 3 or 1 mg/l in row 1 of the test dish failed to reveal any differences in dose response between test isolates.

Figure 4. Diagram illustrating responses of 45 isolates to fenpropimorph and fenpropidin.

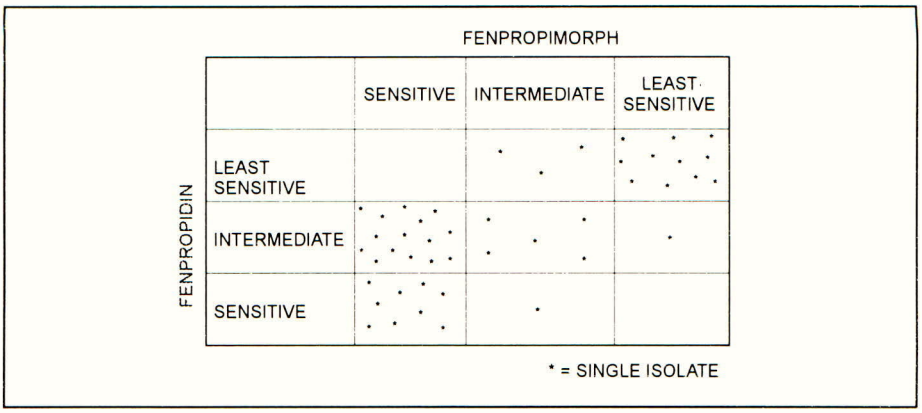


Figure 4 displays the responses of the isolates to fenpropidin and fenpropimorph. It is clear that the distribution of the isolates in this matrix is such that there is a positive correlation between the sensitivities of isolates to these two chemicals. In the absence of such a correlation, the spread of isolates would be random across the grid, with no noticeable clusters in any of the boxes.

CONCLUSIONS

Compared with 1991/1992, the 1993 survey detected a shift towards decreased fenpropimorph sensitivity in the wheat powdery mildew population in all regions of Northern France. The frequency of isolates classified here as 'least sensitive' to fenpropimorph has doubled since 1991. It must be emphasised, however, that no attempt has been made to relate these results to the field performance of fenpropimorph, which remains good (Russell, 1993).

Fenpropimorph acts as a strong inhibitor of sterol Δ^{14} reductase in the sterol biosynthesis pathway. It also has a weaker effect on $\Delta^{8} \rightarrow \Delta^{7}$ isomerase (Baloch *et al.* 1984, Berg *et al*, 1984). As with other sterol biosynthesis inhibitors, the evolution of resistance to morpholines may be a multi-step process, under the control of several genes or alleles. Under continual selection pressure, and in the absence of effective anti-resistance strategies, isolates with increasingly low levels of sensitivity may become more frequent in the population as time progresses. The existence of three different sensitivity classes, as reported here, appears to support this theory. Similar shifts in sensitivity have

been reported in previous surveys in Germany and Switzerland (Lorenz *et al.* 1992).

Fenpropidin, (a piperidine), has a similar mode of action to fenpropimorph, and consequently, the possibility of the development of 'cross-resistance' between fenpropimorph and fenpropidin has been considered (Brown & Evans, 1992). This phenomenon occurs when the response of isolates to one fungicide is positively correlated with the response to one or more others. In our survey, there was a marked tendency for isolates with decreased sensitivity to fenpropimorph to also exhibit reduced sensitivity to fenpropidin. This suggests that the sensitivities of *E. graminis* f.sp. *tritici* isolates to these two fungicides are positively correlated. No such relationship was established between tridemorph (another morpholine) and either of the above chemicals (data not shown). All the isolates tested exhibited a similar response to tridemorph, despite over 20 years of continuous usage of this fungicide in France.

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THE EFFECT OF REDUCED DOSES ON THE SENSITIVITY OF POWDERY MILDEW TO FENPROPIMORPH IN BARLEY FIELD TRIALS

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ABSTRACT

Three field experiments were carried out in 1992 and 1993 to study the effect of reduced doses of fenpropimorph alone, or mixed with propiconazole, on the sensitivity of barley powdery mildew *Erysiphe graminis* f.sp. *hordei*. There were no significant differences between the full commercial dose and reduced doses in their effects on the sensitivity of powdery mildew to fenpropimorph. The sensitivity of the experimental isolates fell within the same range of EC_{50} values as that found in previous sensitivity tests with fenpropimorph. It is is concluded that fenpropimorph is still effective in controlling barley powdery mildew.

INTRODUCTION

Considerable efforts have been directed towards breeding barley for resistance to powdery mildew caused by *Erysiphe graminis* f.sp. *hordei*. Although the use of resistant cultivars has reduced infection levels, changes in the virulence spectrum of the pathogen population present problems to the breeder. The disease remains important in barley growing and farmers must continue to rely for control on the application of fungicides. Genetic variation in the mildew population again gives rise to problems, in this case with respect to the level of sensitivity to fungicides. After the development of resistance to some of the early systemic fungicides used for barley mildew control, the demethylation inhibitors (DMI, azoles) and aminopyrimidine (ethirimol) (Fletcher & Wolfe, 1981; Wolfe, 1985; Heaney, Martin & Smith,1988), control of powdery mildew has relied almost exclusively on one class of fungicides. Commonly referred to as the morpholines, the group consists of two morpholines, fenpropimorph and tridemorph, and a piperidine - fenpropidin. These fungicides are marketed commercially as the individual active ingredients, as well as in mixtures with each other or other compounds.

With such heavy reliance for control on one group of fungicides, selection pressure on the mildew population must be significant. Brown and Evans (1992) described isolates that were resistant to reduced doses of tridemorph, fenpropidin and fenpropimorph, and in addition reported cross resistance between fenpropimorph and fenpropidin. They indicated, however, that the levels of resistance they found were unlikely to cause a substantial loss of effectiveness of the chemicals immediately after spraying.

The Scottish Agricultural College at Edinburgh has been monitoring the sensitivity to fenpropimorph of isolates of barley powdery mildew, collected mostly from Eastern Scotland but also from other parts of Britain, since 1988. The results of this survey show that over this period the mean sensitivity of isolates tested to fenpropimorph has changed little, and EC_{50} values for different years fall within the same range as shown in Table 1. There were however significant differences in the mean sensitivity of isolates between seasons, demonstrating that the population is not stable in terms of sensitivity to morpholines.

Table 1. Sensitivity to fenpropimorph in isolates of powdery mildew collected from 1988 to 1992

	Sensitivity to Fenpropimorph mean EC_{50} value in g/l		
Year	Mean	Range	SED
1988	0.057	0.007 - 0.119	±0.0118
1989	0.021	0.010 - 0.051	±0.0023
1990	0.033	0.008 - 0.115	±0.0061
1991	0.082	0.010 - 0.119	±0.0189
1992	0.029	0.010 - 0.108	±0.0140

Many farmers use reduced doses of morpholines, usually in mixtures, as standard practice to control mildew infections. Because of the possibility that such practices might influence the selection pressure for insensitive isolates in the mildew population, field experiments were carried out to determine if the use of reduced doses was likely to influence the sensitivity of powdery mildew to fenpropimorph, the most commonly used morpholine.

This paper reports the results of three field experiments carried out in 1992 and 1993 to establish if any shift in sensitivity could be measured following a repeat application of reduced doses as applied in standard practice.

MATERIALS AND METHODS

In the spring of 1992, a large field experiment was laid out at Boghall Farm, at Bush Estate in the Lothian Region of Scotland. The barley cultivar used was Golden Promise. Plot sizes were 24m by 16m. Fertiliser, herbicide and any micro-nutrient treatments were uniform across all plots, and accorded with local practice. Seed for the trial was treated with a single purpose seed treatment only (mercury).

Fungicide treatments consisted of two spray programmes of fenpropimorph alone or in a mixture with propiconazole. The first fungicide application was made when mildew first developed on the plants and the second spray was applied three weeks later. There were eight treatments, shown in Table 2, and three replicates of each laid out in blocks. To facilitate spraying plots within blocks were not completely randomised.

Table 2. Fungicide programmes evaluated in 1992 field experiment

Treatment	First application	Second application
U	nil	nil
A	fenpropimorph 1.0*	fenpropimorph 1.0
B	fenpropimorph 0.5	fenpropimorph 0.5
C	fenpropimorph 0.25	fenpropimorph 0.25
D	fenpropimorph 1.0 +propiconazole 0.5	fenpropimorph 1.0 +propiconazole 0.5
E	fenpropimorph 0.5 +propiconazole 0.25	fenpropimorph 0.5 +propiconazole 0.25
F	fenpropimorph 0.25 +propiconazole 0.125	fenpropimorph 0.25 +propiconazole 0.125
G	nil	fenpropimorph 0.25 +propiconazole 0.125

* dose rates as a proportion of the full commercial dose of the products used: full commercial doses for the products used were as follows:

Active ingredient	Product	g AI / ha
fenpropimorph	Corbel	750
propiconazole	Tilt 250 EC	125

All fungicides were applied using a tractor mounted Allman hydraulic sprayer with standard flat fan nozzles in 270 l / ha of water at a pressure of 2 bars

Infected leaves were sampled from the middle of plots at three times during the season; before spraying and three weeks after both the first and second sprays. Isolates from leaves from each plot were tested for sensitivity to fenpropimorph in the laboratory following the method reported in detail by Robertson *et al.*, (1990). Isolates were cultured on detached leaf segments of Golden Promise and maintained on Davis minimal medium containing 80 mg/l benzimidazole. To determine the sensitivity of isolates in tests, seedlings of Golden Promise were grown to the two leaf stage and then fenpropimorph solutions applied at concentrations of 0.015, 0.029, 0.058, 0.117 and 0.234 g AI / l in a spray cabinet using a Humbrol spray gun for five seconds. Control plants were sprayed with water. Each spray treatment was repeated in the same cabinet for replication. Segments of the treated leaves were then plated on the minimal medium and inoculated with the experimental isolates. The mildew cover after 14 days incubation at 18°C was analysed using a Genstat 5 programme which allowed EC_{50} values to be calculated.

Following the field methodology described, two further experiments were laid out at separate sites at Bush Estate in the spring of 1993 but only fenpropimorph was sprayed at full and at three reduced doses as shown in Table 3. To reduce uncontrolled variation brought about by freely mobile inoculum in untreated plots, there were no

unsprayed plots. There were three replicates of each of the four treatments, laid out as before. Plot sizes were 24m by 17m. The seed was treated with guazatine plus imazalil.

Table 3. Fungicide programmes evaluated at two sites in 1993

Treatment	First application	Second application
A	Fenpropimorph 1.0*	Fenpropimorph 1.0
B	Fenpropimorph 0.75	Fenpropimorph 0.75
C	Fenpropimorph 0.5	Fenpropimorph 0.5
D	Fenpropimorph 0.25	Fenpropimorph 0.25

* dose rate as a proportion of the full commercial dose of fenpropimorph as follows:

Active ingredient	Product	g AI / ha
fenpropimorph	Corbel	750

Sampling of infected leaves for tests for sensitivity to fenpropimorph in the laboratory were carried out as in 1992.

RESULTS

The results for the three field experiments are summarised in Tables 4 and 5. Although the sampled isolates varied in their sensitivity to fenpropimorph there were no significant differences between mean EC_{50} values for isolates in relation to the concentration of fungicide to which they had been exposed.

For samples assessed after the second spray application in 1992 (Table 4), the untreated isolates showed an EC_{50} value of 0.056 g/l while those exposed to fenpropimorph alone showed comparable values of 0.032 for the highest dosage rate and 0.025 for the lowest. Where fenpropimorph was combined with propiconazole the equivalent values were 0.023 and 0.086.

Table 4. Sensitivity of isolates from 1992 experiment to fenpropimorph based on mean EC_{50} values in g/l

Sampling time	Fungicide treatments								
	U	A	B	C	D	E	F	G	SED
Before spray	0.092	0.05	0.111	0.001	0.128	0.048	0.244	0.096	-
After 1 spray	0.034	0.035	0.102	0.055	0.118	0.029	0.139	0.077	±0.0470
After 2 sprays	0.056	0.032	0.086	0.025	0.023	0.073	0.136	0.086	±0.0636

In 1993, when fenpropimorph was applied alone at full or reduced rates to all plots at two sites (Table 5), the EC_{50} values at site 1 after the second spray application ranged from 0.120 for the isolates from plots receiving the highest dosage rate to 0.030 for those receiving the lowest. At site 2 the equivalent values were 0.015 and 0.011.

Table 5. Sensitivity of isolates from 1993 experiments to fenpropimorph based on mean EC_{50} values in g/l

Site	Sampling time	Fenpropimorph treatments				
		A	B	C	D	SED
1	Before spray	0.068	0.023	0.111	0.070	
	After 1 spray	0.029	0.181	0.102	0.156	
	After 2 spray	0.120	0.081	0.087	0.030	±0.0695
2	Before spray	0.027	0.102	0.029	0.188	
	After 1 spray	0.059	0.115	0.031	0.012	
	After 2 sprays	0.015	0.038	0.036	0.011	±0.0574

In considering the variation in sensitivity between times of sampling, there were no significant differences between the mean EC_{50} values for isolates sampled before any spray application, after one application of fenpropimorph or after two applications, in both seasons. In 1992, the mean EC_{50} of all the isolates collected before any sprays were applied was 0.096. After the first spray the mean was 0.064 for all treatment plots that had received fenpropimorph alone and 0.095 for those that received fenpropimorph plus propiconazole. After the second spray the comparable values were 0.049 and 0.077. In 1993 the mean EC_{50} for all isolates collected from treatment plots before spray application at site one was 0.068 and at site two was 0.086. After one spray the mean values were 0.117 (site 1) and 0.054 (site 2) and after the second spray 0.080 and 0.025 respectively.

DISCUSSION

There was no evidence from the field experiments conducted in 1992 and 1993 that variation in dose rates of fenpropimorph affected the level of sensitivity of isolates of barley powdery mildew exposed to this fungicide within two growing seasons. There was also no significant difference between the sensitivity of untreated plots in 1992 and those which had received fenpropimorph sprays. The range of sensitivities of the isolates tested in all three trials fell within the range found during routine monitoring from 1988 onwards. Brown and Evans (1992) also reported that they could find no

correlation between dose rate and frequency of insensitivity, although they did report an increased frequency of resistance after exposure to fenpropimorph sprays. They concluded that the reductions in sensitivity that they observed were unlikely to result in any reduction in field performance.

Reduced rates of morpholine clearly still provide effective control of mildew in the field situation (Wale et al., 1993). The results of the three trials reported here, where reduced doses of morpholine were applied twice in the season as is common agricultural practice confirm the observations of farmers and advisers that reduced doses have not significantly affected the performance of fenpropimorph against the pathogen. Further trials over several years, however would be necessary to establish the long term effects of reduced dose rates on fenpropimorph sensitivity in fungal populations.

In conclusion there was no evidence that reduced doses of fenpropimorph, applied following normal agricultural practices, are likely to reduce the sensitivities of mildew isolates in treated plots. In keeping with previous observations, fenpropimorph was found to retain its effectiveness as a fungicide for the control of barley powdery mildew in the field.

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