Session 4
Morpholines

Chairman	J GILMOUR
Session Organiser	D W HOLLOMON

DO MORPHOLINE FUNGICIDES SELECT FOR RESISTANCE?

D.W. HOLLOMON

Department of Agricultural Sciences, University of Bristol, Institute of Arable Crops Research, Long Ashton Research Station, Long Ashton, Bristol BS18 9AF, UK.

ABSTRACT

Morpholine fungicides are considered as low risk for the development of resistance and have been used for more than 25 years without performance difficulties. Fundamental studies on both the morpholine and piperidine fungicides in this group revealed that they are multisite inhibitors of sterol biosynthesis. Resistance can be generated in the laboratory, but is under polygenic control. Variation in sensitivity in field populations can be detected by a variety of assay techniques, and some selection for reduced sensitivity occurs following treatment. Although seasonal short-term changes in morpholine sensitivity can occur, evidence for a long-term decline in sensitivity is not convincing, and no loss of field performance has been reported.

INTRODUCTION

Morpholines are an important group of systemic fungicides especially useful in controlling powdery mildew diseases on a wide range of crops. Chemically, they are somewhat diverse and include both morpholines (tridemorph, dodemorph, aldimorph, fenpropimorph, Figure 1), and piperidines (piperalin, fenpropidin). The first morpholine introduced was piperalin (Piperonil) in 1960 for control of rose powdery mildew (*Sphaerotheca pannosa*) in protected rose production; it is still in use today without any apparent loss in efficacy (Arnold W., personal comm., 1994). Rose mildew was also the target for dodemorph (Meltatox), but tridemorph (Calixin, Bardew), fenpropimorph (Corbel, Mistral) and fenpropidin (Patrol) have all been used extensively for cereal mildew control. Apart from one isolated report of tridemorph resistance (Walmsley-Woodward *et al.*, 1979) the field performance of morpholines has remained good. Consequently, morpholines are important components, often at reduced dose rates, in mixtures designed to combat the spread of resistance to the sterol 14 demethylase inhibiting (DMI) fungicides (Heaney *et al.*, 1988).

A strong feature of morpholine action against powdery mildews is their rapid "knockdown" action, but, in Northern France especially, there have been suggestions that this action has weakened and more frequent applications, particularly of fenpropimorph, are now needed to achieve a high level of control of wheat powdery mildew (*Erysiphe graminis* f.sp. *tritici*). In addition, there have been reports of shifts in sensitivity to fenpropimorph in France (Andrivon *et al.*, 1987), the Netherlands (De Waard, 1992), and Germany and Switzerland (Lorenz *et al.*, 1992). In the absence of new fungicides with novel modes of action, any development of practical resistance to morpholines would be

of great concern in disease control. This paper examines factors of morpholine action which influence the risk of resistance occurring, and reviews evidence available before this meeting, that morpholine resistance is a practical problem.

MODE OF ACTION

Despite several false starts, work on *Botrytis cinerea* and *Ustilago maydis* finally established that tridemorph inhibited sterol biosynthesis at two steps in the pathway, Δ^{8-7} isomerase and Δ^{14-15} reductase (Table 1). These conclusions were later confirmed using cell-free extracts from yeast (See Köller (1992) for review on morpholine action). Even so, the action of tridemorph against the Δ^{14-15} reductase was very weak, whilst fenpropidin hardly affected the isomerase. Much depended on the fungus and in barley powdery mildew (*E. graminis* f.sp. *hordei*) not only were the effects on the accumulation of Δ^{8} and Δ^{14} abnormal sterols small, but other sterol changes were observed (Senior, 1991). Inhibition of $\Delta^{24(28)}$ reductase, Δ^{24} transmethylation, and squalene cyclisation have all been identified as steps inhibited by morpholines (Ziogas, *et al.*, 1991; Schneegurt and Henry, 1992), together with possible feedback inhibition of HMG-CoA reductase. A common feature of these target sites is that all involve high energy carbocationic intermediates, which are mimicked by the morpholines because they are protonated at the ring N at physiological pH. Although the sensitivity of these steps may differ, since morpholines are applied at high rates (3.75 - 5.0 ml product litre^{-1}; 3.75 g a.i. litre^{-1}; 13 mM a.i.) all sites are probably inhibited under field conditions. Because of this multisite action it is unlikely that resistant mutants will arise rapidly. Furthermore, the carbon chain attached to the heterocyclic nitrogen in all morpholines is quite flexible, and can adopt several different configurations, making it difficult for the target site protein to alter to exclude the fungicide.

GENETICS OF RESISTANCE

It is not difficult to generate laboratory mutants resistant to morpholine fungicides, and especially fenpropimorph. In both *U. maydis* (Erg 40, Walsh and Sisler, 1982) and yeast (Parks, personal comm., 1994) these do not involve mutations in the target site genes. The practical significance of these mutations is not clear since they show cross resistance with DMI fungicides, a characteristic generally lacking in DMI resistant field strains. Molecular genetic techniques have not only highlighted the possible uniqueness of tridemorph, but have questioned the involvement of Δ^{8-7} isomerase in its action. We have generated targeted mutations in *Neurospora crassa* which are sensitive to tridemorph, but are quite resistant to fenpropimorph and fenpropidin (Hollomon, unpublished observation). Furthermore, an *Ustilago maydis* mutant lacking Δ^{8-7} isomerase activity was able to grow almost as well as its wild-type parent (James *et al.*, 1992), although these experiments provided no measure of the effect of Δ^{8-7} isomerase mutations on pathogenicity. Nevertheless, genetic studies with *Nectria haematococca* var. *cucurbitae* indicated polygenic control of fenpropimorph resistance which would reduce the risk of resistance occurring in field populations (Demakopoulou *et al.*, 1989).

FIGURE 1. Morpholine and piperidine fungicides used in agriculture.

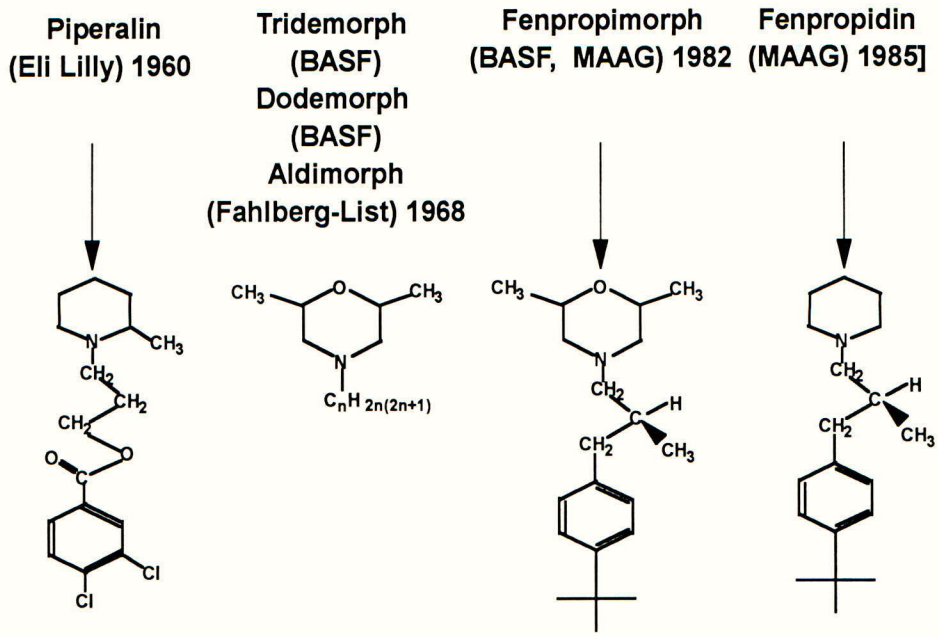

FIGURE 2. Changes in fenpropidin and triadimenol sensitivity in barley powdery mildew 1973-1993.

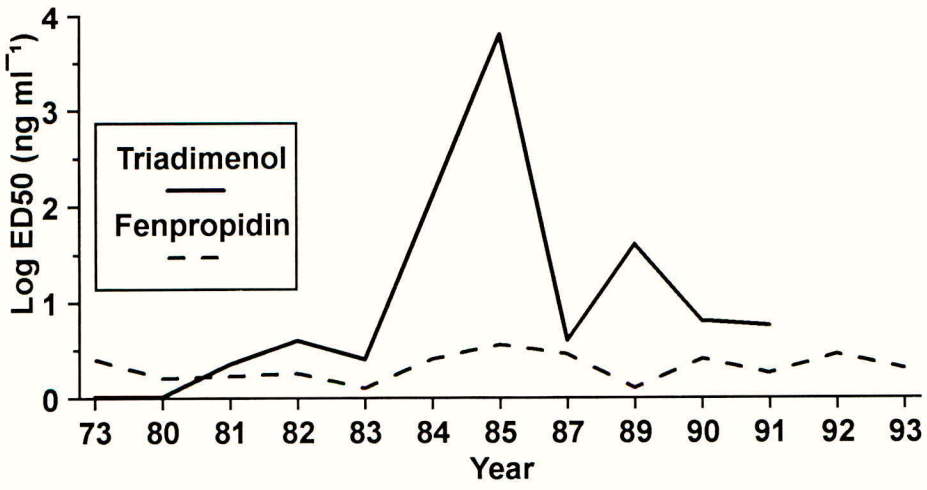

TABLE 1. Inhibition of sterol biosynthesis by morpholines

Δ^{8-7} isomerase	Fenpropimorph, Piperalin, Tridemorph
Δ^{14-15}reductase	Fenpropidin, Fenpropimorph
$\Delta^{24(28)}$ reduction	Fenpropidin, Piperalin
Δ^{24} transmethylation	Fenpropidin
Squalene cyclisation	All
Feed-back Inhibition	?

TABLE 2. Ranking for fenpropidin sensitivity of barley powdery mildew isolates using two different assay methods

	Vapour Test[1]	Detached Leaf Test[2]	ED50 (μg/ml)
Sensitive	L32	1641	(0.01)
	BUSB 22	BUSB 22	
	BUSB 20	CSB 2	
	23D5	BUSB 20	
	CSB 2	23D5	
	DH14	DH14	
Resistant	1641	L32	(0.14)

1 Assay as described by Readshaw and Heaney (1994) except that leaf segments were placed on agar. Fenpropidin was applied to leaf segments.

2 Assay using detached barley leaf segments floating on fungicide solutions as described in Hollomon (1982).

TABLE 3. Changes in Fenpropimorph and Triadimenol sensitivity in wheat powdery mildew.

	Mean ED_{98} (μg ml^{-1} AI)	
Year	Fenpropimorph	Triadimenol
1984	9	22
1986	8	14
1988	4	5
1989	15	26
1990	30	39
1991	9	93

Data from Lorenz and Pommer (1984); Lorenz *et al.*, (1992)

DETECTION OF VARIATION IN SENSITIVITY

Several different assay procedures have been used to measure morpholine sensitivity. Most focussed on cereal powdery mildews, and seedlings have been sprayed, sometimes with upto 14 different dose rates to give meaningful dose/response relationships. Inconsistences in foliar applications arising through different orientations of leaves in relation to the spray nozzle, may be reduced by using root drenches instead. Other monitoring procedures have involved inoculation of treated leaf pieces, placed either on an agar surface or on fungicide containing solutions. All morpholines have some vapour action and this has been exploited for monitoring cereal powdery mildews (Readshaw and Heaney, 1994).

Considerable variation is a component of all assay methods. Brown *et al.* (1991) used **P**rincipal **C**omponent **A**nalysis (PCA) to overcome variability within seedling assays, but this is generally seen as a preliminary statistical procedure requiring further analysis before conclusions can be drawn about differences between sensitivity classes (Arnold, G.M., personal comm., 1993). Direct comparisons between dose rates used in the field and those applied in the laboratory can be misleading, but dose rates generally used in laboratory and greenhouse assays are well below those recommended for field use.

To allow comparisons between test occasions, standard strains of known sensitivity should be included in all assays. Whilst standard strains of known sensitivity to DMIs have given similar rankings in "ring tests", carried out in different laboratories, the same is apparently not the case for standards used in morpholine tests. In our laboratory, tridemorph resistant isolates identified by Walmsley-Woodward *et al.*, (1979) were ranked no different from other isolates (Hollomon, unpublished data) whilst standard strains CC1, CC139 and CC151 (Brown *et al.*, 1991) were within the wild-type variation encountered in natural populations of barley powdery mildew in Scotland (Zziwa, personal comm., 1993). Choice of the wild-type standard sensitive strain must reflect the mean sensitivity of the whole population, and not just the extreme sensitive end of that variation. Fenpropidin sensitivity determined by the vapour test did not rank strains in the same order as an assay using detached leaf segments (Table 2) floating on fenpropidin solutions. This emphasises that morpholine vapour action may not be identical to that exerted on the pathogen through systemic entry from leaf tissue. Despite these limitations, differences in morpholine sensitivity between isolates clearly exist, and can be detected by several different assay procedures. The range of variation in natural cereal mildew populations may well exceed 50-fold.

TABLE 4. Fungicide efficacy: Spring Barley (1980-1992)

	Mean Disease Control (%)			
	Bayfidan	Tilt	Corbel	Patrol
1980	95*	83	83	-
1984	69	45	79	-
1985	55	20	73	70
1988	21	-	70	66
1990	80	85	66	68
1992	75	82	73	76

* Bayleton

Barley powdery mildew

A collection of *E. graminis* f.sp. *hordei* strains have been maintained in our laboratory since 1973. Data are available, therefore, for fenpropidin sensitivity of strains isolated from the field between then and 1993. Although the numbers tested varied from over 300 strains in 1984 to 10 in 1988, and variation associated with the mean ED_{50} for each year differed, no significant changes in fenpropidin sensitivity were observed throughout this period (Figure 2). By contrast, changes in DMI sensitivity were easily identified in the same strains. Whilst there have been differences each year in the mean sensitivity to fenpropimorph of barley mildew isolates collected in Scotland, no consistent decline in sensitivity was observed over the period 1988-1992 (Zziwa and Burnett, 1994). This contrasts with results of other surveys carried out in Scotland, where significant differences in fenpropimorph sensitivity were detected between 1988 and 1990. But this may simply reflect regional differences arising from clonal selection for reduced sensitivity (Brown *et al.* 1991; Brown and Evans, 1992). Changes in morpholine sensitivity have also been detected within field experiments (Brown and Evans, 1992), but were not correlated with performance changes and may just represent the sensitivity of the surviving small population.

Wheat powdery mildew

A decline in fenpropimorph sensitivity in *E. graminis* f.sp. *tritici* was observed in several parts of Europe between 1988 to 1990 (DeWaard, 1992; Lorenz *et al.*, 1992; Felsenstein, 1991). Changes were not large when compared to the decline in DMI sensitivity that occurred at the same time (Table 3). As with barley powdery mildew, changes in sensitivity appear seasonal. Any decline in fenpropimorph sensitivity observed in Germany in 1990 was reversed the following year (Table 3), when the mean sensitivity of the population returned to the level in 1984. These shifts in fenpropimorph sensitivity were not, it seems, correlated with field performance.

FIELD PERFORMANCE

Yearly variation in field and crop conditions make it difficult to evaluate long term changes in fungicide performance. Because of the vapour activity of morpholines, temperature at the time of spraying can very much influence performance. Comparisons based on the same cultivar and disease levels over several years are seldom available, but the results in Table 4 attempt to combine performance data against barley powdery mildew from a number of ADAS and Long Ashton Spring barley field trials, carried out between 1980 and 1992. Conditions favoured good mildew control in 1980, but since then there has been no dramatic fall in the performance of fenpropimorph or fenpropidin. Evidence for tridemorph is more fragmentary, but again shows no clear change in performance since Calixin was introduced some 25 years ago. This contrasts with the decline in the performance of early triazoles (DMIs) against cereal mildews, which is easily seen alongside the morpholine results (Table 4). In France, it seems that the performance of fenpropimorph against wheat powdery mildew may have weakened in some regions (Maumené, personal comm., 1994), but not that of fenpropidin. With the exception of black Sigatoka disease (*Mycosphaerella fijiensis*) on bananas (Cronshaw *et al.*, 1994), for other diseases where morpholines play a part in disease control strategies, long term performance results are not generally available.

DISCUSSION AND CONCLUSION

Morpholine fungicides have always been considered a low risk for resistance. In part this is based on practical experience, since both piperalin and tridemorph have been used now for more than 25 years without noticeable changes in performance. They have certainly been used in situations where resistance to other groups of fungicides, i.e. benzimidazoles, hydroxypyrimidines and triazoles, has developed rapidly. Work on mode of action has identified morpholines as multisite inhibitors of sterol biosynthesis, and highlighted differences between fenpropidin/fenpropimorph and tridemorph, which are reflected in cross-resistance patterns. Laboratory mutants resistant to morpholines are easy to generate in several fungi, indicating that the biochemistry required to overcome the effects of morpholines is possible. But the exact mechanisms of resistance are unknown, although morpholines are bulky molecules with flexible side chains which may be difficult to accommodate through a change in the target site protein.

It is not surprising that variation in the sensitivity of plant pathogens to morpholines should exist. Despite limitations, several assay procedures have been used to chart the range of this variation, although unfortunately no base-line sensitivity data were collected before morpholines were introduced. Selection of this variation leading to an increase in frequency of field resistant strains is clearly possible, but evidence that changes have caused a decline in field performance is less clear. Indeed, no cases of practical resistance have been reported in the literature for any morpholine fungicide. Field rates may simply be sufficient to control populations with reduced sensitivity. Furthermore, where there have been suggestions that mildew control has not lasted so long after spraying, these reports have centred around fenpropimorph and not fenpropidin, although laboratory studies show cross resistance exists between these two fungicides. It is fortunate that practical resistance to morpholines has not occurred,

otherwise control of cereal powdery mildews would be difficult without new fungicides, with novel modes of action to replace them.

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THE GENETICS OF THE RESPONSE OF BARLEY MILDEW TO MORPHOLINE AND PIPERIDINE FUNGICIDES

JAMES K.M. BROWN

Cereals Research Department, John Innes Centre, Colney Lane, Norwich, NR4 7UH, England

ABSTRACT

Responses of the barley powdery mildew pathogen, *Erysiphe graminis* f.sp. *hordei*, to three fungicides were studied. Two of these chemicals were morpholines, fenpropimorph and tridemorph, and a third, fenpropidin, was a piperidine. Three classes of *E.g.* f.sp. *hordei* isolate were found. One type was sensitive to all three chemicals, another was resistant to fenpropidin and fenpropimorph, while a third had further resistance to fenpropimorph and also some resistance to tridemorph. The levels of resistance observed were not high enough to have caused serious failures of mildew control. The use of either fenpropidin or fenpropimorph in field trials selected isolates which were resistant to both fungicides, while tridemorph selected against fenpropidin resistance. A single gene controlled responses to these three fungicides, but had no effect on the response to a demethylation inhibitor (DMI) fungicide, triadimenol.

INTRODUCTION

Morpholine and piperidine fungicides have been widely used to control powdery mildew of barley (*Erysiphe graminis* f.sp. *hordei*). The most important of these chemicals are a piperidine, fenpropidin, and two morpholines, fenpropimorph and tridemorph. All three inhibit the synthesis of ergosterol from lanosterol (Baloch *et al.*, 1984).

Reduced sensitivity of *E.g.* f.sp. *hordei* to tridemorph was first reported by Walmsley-Woodward *et al.* (1979), and to fenpropimorph by Wolfe *et al.* (1987). Following continuing reports of diminishing sensitivity (Wolfe *et al.*, 1988), more extensive surveys of the responses of *E.g.* f.sp. *hordei* to these fungicides, and to fenpropidin, were undertaken in 1988 and 1990. This paper reviews this work. Three aspects are considered: (i) the phenotypes of resistance to the three fungicides; (ii) selection for resistance in field trials; (iii) the genetic control of responses to these fungicides.

RESISTANCE AND CROSS-RESISTANCE

In 1988, a national survey of *E.g.* f.sp. *hordei* isolates was undertaken. This was described in detail by Brown *et al.* (1991b). Random samples of airborne spores were collected by a wind-impaction spore trap mounted on a car (Wolfe *et al.*, 1981). In England, samples were obtained from Lincolnshire, Cambridge, Suffolk and Essex. In Scotland, samples were collected along a route which went north from Newcastle to Edinburgh, Perth and Aberdeen, then made a circular tour through Banffshire and Moray, and then returned from Aberdeen to Newcastle.

Single colony isolates were tested for their responses to fenpropidin, fenpropimorph and tridemorph. Isolates from England showed no significant variation in responses to these fungicides, all being similar to isolates collected before morpholines and piperidines came into widespread use.

Isolates from Scotland, however, were polymorphic. In tests of responses to fenpropidin, all isolates fell into one of two distinct groups, and were thus classified as either sensitive or resistant. Responses to fenpropidin were strongly correlated with those to fenpropimorph, in that all isolates which were resistant to fenpropidin had a higher level of resistance to fenpropimorph than fenpropidin-sensitive isolates did. In all, 39 out of 73 Scottish isolates were resistant.

Sensitive isolates, whether from England or Scotland, did not differ significantly in their responses to tridemorph from those of most of the resistant isolates. However, four isolates, all from Moray, showed a three-fold increase in resistance to fenpropimorph over that of the rest of the fenpropidin-resistant class. Furthermore, the sensitivity of these four to tridemorph was halved, compared to that of most other isolates. Three of these four samples were studied further, by virulence tests of ten differential varieties of barley. All of them proved to have the same virulence phenotype. This suggests that this group was genetically homogeneous, and was perhaps a clone.

Three phenotypes of *E.g.* f.sp. *hordei* were therefore observed. One, sampled in both England and Scotland, had baseline sensitivity to fenpropidin, fenpropimorph and tridemorph (phenotype S). Another, found in Scotland, had resistance to fenpropidin and fenpropimorph, but not tridemorph (phenotype R). A third, only found in Moray, in north-east Scotland, had a further increase in fenpropimorph resistance and also had some tridemorph resistance (phenotype RM). However, the resistance of types R and RM were sufficiently low that barley mildew would still have been controlled by either fenpropidin or fenpropimorph.

SELECTION ON RESISTANCE

From the results of the survey in 1988 (Brown *et al.*, 1991b), it was predicted that, in the field, fenpropidin and fenpropimorph would select for resistance to each other, while neither would select for resistance to tridemorph, and that tridemorph would not select for resistance to fenpropidin or fenpropimorph. These predictions were tested in 1990. The experiments were described by Brown & Evans (1992).

Six field trials were carried out, two in England and four in Scotland. In each trial, thirteen different spray treatments were applied to 12m × 3.2m plots in a field of a mildew-susceptible barley cultivar, Golden Promise. These were replicated in two blocks at each site, and the full trial of 26 plots was replicated at the six locations. In each block, one plot was sprayed with water, and four each were sprayed with fenpropidin (Patrol), fenpropimorph (Corbel) or tridemorph (Calixin). For each fungicide, the four treatments were applied at the full recommended rate, or at a half, a quarter or an eighth of that rate. (The full rates are 1.0 l ha^{-1} of Patrol and Corbel and 0.7 l ha^{-1} of Calixin, with a concentration of 750 g l^{-1} of the active compound in each case. Fungicides were applied in 250 l ha^{-1} water.)

Samples of *E.g.* f.sp. *hordei* were obtained from each site except for one of the English ones, which was too lightly infected with mildew for sampling to be worthwhile. Up to 20 single colony isolates were obtained from each plot. Isolates from untreated plots at three sites in Scotland, and at the remaining English site, were put through a preliminary screen for resistance to fenpropimorph. All of the Scottish isolates, and some English isolates, were resistant. Clearly, the frequency of resistance had increased between 1988 and 1990.

Isolates from a site in north-east Scotland, Banff, and from one in eastern England, Levington, in Suffolk, were studied more intensively. The doses used to analyse the 1990 isolates were more closely spaced, and covered a greater range, than those used in 1988. Also, a numerical scale, the minimum inhibitory concentration, was used to analyse responses in 1990, whereas most of the analysis of the 1988 results was done by simply classifying the responses of isolates to each fungicide as resistant or sensitive. More detailed comparisons could therefore be made between responses to the different fungicides. The results are shown in detail in Figure 1.

No polymorphism was observed at Banff, all isolates tested being resistant to both fenpropidin and fenpropimorph. At Levington, as in 1988, there was a strong correlation between responses to fenpropidin and fenpropimorph, such that isolates could be classified as resistant to both, or as sensitive.

The mean resistance to fenpropidin of Banff isolates was the same as that of the resistant isolates from Levington, both being about 25 times more resistant than sensitive Levington isolates.

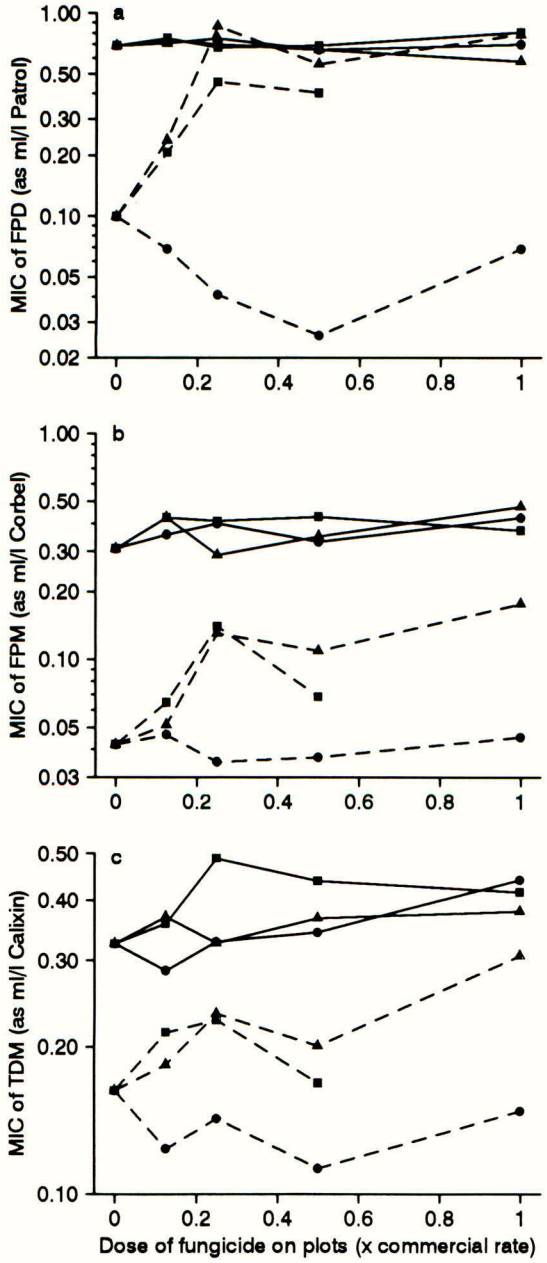

FIGURE 1. Mean responses to (a) fenpropidin (FPD), (b) fenpropimorph (FPM) and (c) tridemorph (TDM) of isolates of *Erysiphe graminis* f.sp. *hordei* sampled from field trial plots treated with each of these fungicides, at Banff, Scotland and Levington, England. (No isolates were obtained from the plots treated with the full rate of FPM at Levington.) The minimum inhibitory concentrations (MICs) shown in ml l^{-1} should be multiplied by 0.24 to calculate MICs in l ha^{-1}. In laboratory tests, fungicides were applied to 10-day-old seedlings of the barley variety Golden Promise in 240 l ha^{-1} water using a hand-held pressurised sprayer with an flat-fan nozzle on a single nozzle lance at 3×10^5 Pa. The concentrations of the active compounds were all 750 g l^{-1} in Patrol (FPD), Corbel (FPM) or Calixin (TDM). Eleven doses of each fungicide were used, covering a range of 4.25×10^{-3} to 2.0 ml l^{-1} for Patrol, 2.13×10^{-2} to 1.0 ml l^{-1} for Corbel and 7.47×10^{-3} to 3.5 ml l^{-1} for Calixin. The ratios of successive doses were 1.85 in all cases. Plants sprayed with water only were used as controls. Further details of the methods used are given by Brown & Evans (1992).

At Levington, isolates which were resistant to fenpropidin were four to five times more resistant to fenpropimorph than sensitive isolates were, while Banff isolates showed a further doubling of fenpropimorph-resistance over the level of the resistant Levington isolates. Furthermore, the Banff isolates were half as sensitive to tridemorph as the resistant Levington isolates. The Banff isolates varied in their responses to fenpropimorph and tridemorph, some being repeatably more resistant than others. All but one of the twelve most resistant Banff isolates had the same set of virulences

as the three more fenpropimorph-resistant isolates from Moray sampled in 1988, the odd one differing by only a single virulence. The twelve least resistant isolates from Banff, however, had diverse virulence phenotypes, as did twelve resistant and twelve sensitive isolates from Levington.

This evidence is consistent with most *E.g.* f.sp. *hordei* at Banff being members of the same clone as that which was sampled in Moray in 1988, having phenotype RM, but with a small fraction having phenotype R. It is also consistent with resistant *E.g.* f.sp. *hordei* at Levington having phenotype R and sensitive isolates being of type S.

Since there was no polymorphism for responses to fenpropidin or fenpropimorph at Banff, neither fungicide selected for resistance either to itself or to the other. At Levington, however, both fungicides selected for resistance to each other, since responses to the two were highly correlated.

Application of each of the three fungicides, fenpropidin, fenpropimorph and tridemorph, selected for greater resistance to tridemorph at Banff, although the increase was not large. If the hypothesis concerning the phenotypes of *E.g.* f.sp. *hordei* at Banff, described above, is correct, the pattern of selection for responses to tridemorph is consistent with type RM isolates being selected in plots treated with higher doses of fungicide.

At Levington, treatment with fenpropidin and fenpropimorph also selected weakly for increased resistance to tridemorph. This suggests that type R isolates may have reduced sensitivity to tridemorph. Application of tridemorph, however, had the unexpected effect of selecting *against* resistance to fenpropidin and, to a lesser extent, fenpropimorph and tridemorph. Selection for increased sensitivity was most marked in plots treated with a quarter or a half of the full dose of tridemorph. This suggests that the fitness of type R isolates is lower than that of type S in the presence of tridemorph.

No higher level of resistance was observed in 1990 than in 1988, although resistance had spread to England and was evidently much more common in Scotland. However, the results from the tridemorph-treated plots at Levington suggested an intruiging possibility for crop protection, in that an early spray with tridemorph might select out type R isolates, and thus improve the efficacy of a later spray with fenpropidin or fenpropimorph. Clearly, a proposition such as this, based on a single trial, must be followed up by proper tests in the field.

GENETICS OF RESISTANCE

The results of Brown *et al.* (1991b) and Brown & Evans (1992) suggested that resistance to fenpropidin and fenpropimorph might be controlled by the same gene or genes, while the additional decrease in sensitivity to fenpropimorph and fenpropidin in type RM isolates might be under common genetic control. These predictions were tested by analysing the progeny of genetic crosses. The results are outlined here and by Brown *et al.* (1992a), and will be described in detail elsewhere (J.K.M. Brown, S. Le Boulaire, N. Evans, in preparation).

In a cross of a type R isolate of *E.g.* f.sp. *hordei* with one of type S, resistances to fenpropidin and fenpropimorph co-segregated in all 55 progeny tested. There was no significant correlation of the responses to these two fungicides with that to tridemorph. The segregation ratio of resistance and sensitivity was not simple, however, since there was a large excess of resistant progeny. It is not known whether this was because resistance was controlled by a more complex genetic system than a single gene, or because factors affecting fitness were linked to the resistance gene.

Resistance to fenpropidin and to fenpropimorph also co-segregated in a cross of a type RM isolate with the same type S isolate. Resistant progeny were more resistant to tridemorph than sensitive progeny were, and were also more resistant to fenpropimorph than were resistant progeny of the type R × type S cross. The segregation ratio was not significantly different from 1:1, and was therefore consistent with the responses to all three fungicides being controlled by a single gene. This gene was not linked to one controlling responses to a sterol C14-demethylation inhibitor (DMI)

fungicide, triadimenol.

DISCUSSION

Brown *et al.* (1991b) and Brown & Evans (1992) showed that resistance to morpholine-type fungicides had developed in the British population of *E.g.* f.sp. *hordei* between 1986 and 1990. The resistance of R and RM isolates was considerably greater than that of any isolate collected before 1986 (Wolfe *et al.*, 1987).

It has also been shown that a piperidine fungicide, fenpropidin, falls into the same cross-resistance group as a morpholine, fenpropimorph. However, a second morpholine, tridemorph, displays little cross-resistance with these two. The pattern of cross-resistance may be associated with the mode of action of these compounds. Although in yeast, all three inhibit two steps in sterol biosynthesis, tridemorph inhibits $\Delta^8 \rightarrow \Delta^7$ isomerisation better than Δ^{14} reduction, while the reverse is true for fenpropidin and, to a lesser extent, for fenpropimorph (Baloch *et al.*, 1984). It can be hypothesised that type R and RM isolates are both less sensitive to inhibition of Δ^{14} reduction than type S isolates are, while type RM isolates also have reduced sensitivity to inhibition of $\Delta^8 \rightarrow \Delta^7$ isomerisation. Despite the complexity of the cross-resistance relationships of these fungicides, responses of *E.g.* f.sp. *hordei* to these chemicals were under simple genetic control in one cross, as are those to ethirimol and to a DMI, triadimenol (Brown *et al.*, 1992b). Whether the genetics of resistance to fenpropidin and fenpropimorph are more complex in the R × S cross is not yet known.

The increase in resistance to fenpropidin and fenpropimorph up to 1990 was probably caused by heavy usage of these fungicides to control mildew. Virulence tests showed that phenotype R was selected in many different genetic backgrounds (Brown & Evans, 1992). Similarly, resistance to triadimenol and to ethirimol occurred in many different clones of *E.g.* f.sp. *hordei* (Brown *et al.*, 1990). This is probably because many clones infect fields of susceptible barley varieties, and are thus selected for increased resistance by applications of morpholine-type fungicides. No definite explanation can be advanced for the high frequency of one particular clone, with phenotype RM, in north-east Scotland (Brown *et al.*, 1991b; Brown & Evans, 1992). However, clones of *E.g.* f.sp. *hordei* have initiated epidemics of mildew on previously resistant varieties (Brown *et al.*, 1990, 1991a). It is possible that type RM mutants are comparatively rare, and that one such clone was selected by the very high use of morpholine-type fungicides on the mildew-susceptible variety Golden Promise, which was widely grown in north-east Scotland.

The future course of resistance to these fungicides in *E.g.* f.sp. *hordei* cannot be predicted. Although successively higher levels of DMI resistance were selected during the 1980s (Brown & Wolfe, 1991), there is no evidence for significant increases in resistance to morpholine-type fungicides since 1990, even from the relatively low levels seen then (Mitchell & Slater, 1994). This may be because isolates with higher levels of resistance are unfit, or, perhaps more likely, because selection for resistance has been relaxed. Since 1990, many more spring barley varieties with an effective mildew resistance gene, *mlo*, have been grown (Mitchell & Slater, 1993), and the use of all fungicides to control barley mildew, including morpholine-type compounds, appears to have fallen markedly.

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