PARASEXUAL ANALYSIS OF MORPHOLINE AND TRIAZOLE RESISTANCE IN *PSEUDOCERCOSPORELLA*

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ABSTRACT

Parasexual crosses between the W and R pathotypes of *P. herpotrichoides* and between *P. herpotrichoides* (R-type) and the related *P. anguioides* were used to investigate the inheritance of resistance to the morpholine fungicide fenpropimorph and the triazoles, difenconazole and triadimenol. Both crosses indicated multiple genes involved in fenpropimorph resistance. A gene (or genes) having a major effect on triazole sensitivity was observed in both crosses with additional genes modifying the level resistance expressed. However, the patterns of cross-resistance seen differed between the two crosses suggesting that the 'major' genes detected differ in their effect.

INTRODUCTION

The eyespot pathogen, *Pseudocercosporella herpotrichoides*, exhibits considerable natural variation in sensitivity to the ergosterol biosynthesis inhibiting (EBI) fungicides. In general, the R pathotype of the fungus is less sensitive to DMI fungicides than the W pathotype. These differences in fungicide sensitivity are likely to be important selective factors in the population dynamics of the W and R pathotypes.

Interpathotype crosses in *P. herpotrichoides* are possible by manipulation of the parasexual cycle in this fungus. This system enables genetic analysis of agronomically important characters in this fungus, regardless of the sexual compatibility of isolates (Hocart *et al.*, 1993). Crosses between the W and R pathotypes have been used to investigate the genetic basis of variation in sensitivity to DMI fungicides. Previous work had shown that DMI sensitivity was controlled by several genes in *P. herpotrichoides*. One or more of these genes segregated at or shortly after formation of the diploid fusion product following protoplast fusion, and has a major effect on the level of DMI sensitivity shown (McNaughton & Hocart, 1994). Additional genes, segregating later in the parasexual cycle, modified the level of resistance expressed.

As a part of this analysis of the genetic inheritance of fungicide resistance progeny from parasexual crosses between the W and R pathotypes of *P. herpotrichoides* (WxR) (Hocart *et al.*, 1993) were assessed for their sensitivity to the morpholine fungicide fenpropimorph, and the triazole fungicides triadimenol and difenconazole. This paper compares the interpathotype results with a similar assessment made using interspecific hybrid progeny generated between *P. herpotrichoides* and the weakly pathogenic *P. anguioides* (RxA) (Hocart & McNaughton, 1994).

METHODS

Interpathotype parasexual recombinant progeny, recovered from a cross between the W and R pathotypes of *P. herpotrichoides* (WxR) (Hocart *et al.*, 1993), and interspecific hybrid strains produced by parasexual recombination between an R-type isolate of *P. herpotrichoides* and *P. anguioides* (Hocart & McNaughton, 1994), were used in the analysis. In both crosses the parasexual cycle was initiated by protoplast fusion.

Sensitivity to the morpholine fungicide fenpropimorph, and the triazole fungicides difenconazole and triadimenol, was assessed *in vitro*. Colony diameters measured after ten days incubation at 19°C were used to calculate relative growth on a range of fungicide concentrations. These data were plotted against fungicide concentration (Log10 μM) and used to determine ED50 values for each strain. Cross-resistance between fungicides was detected by correlation analysis using the Log10 ED50 data.

RESULTS & DISCUSSION

Interpathotype Cross

There was a six-fold difference in the sensitivity to fenpropimorph between the W-type (ED50 = 9.8 μM) and R-type (ED50 = 63 μM)strains used as the parents in the interpathotype cross (Fig. 1a). Fenpropimorph sensitivity of WxR progeny varied from highly sensitive (ED50 = 0.1 μM) to almost as resistant as the R-type parent (ED50 = 47 μM) (Fig. 1a). Some recombinants were super-sensitive to this fungicide. The range of resistance phenotypes obtained strongly suggest that fenpropimorph sensitivity is determined by multiple genes.

The two sub-groups of WxR progeny, sensitive (●) or resistant (○) to the DMI fungicides are clearly seen in Fig. 1d. The 'major' gene(s), having a major effect on DMI sensitivity, confers cross-resistance between the two DMI fungicides used. Additional genes, modifying the level of resistance expressed, were seen to segregate at a later stage in the parasexual cycle (McNaughton & Hocart, 1994).

No cross-resistance was observed to fenpropimorph which could be attributed to the 'major gene(s)' for DMI resistance (Figs. 1b &1c). However, there was some evidence that the 'minor genes' could confer cross-resistance between fenpropimorph and triadimenol, subject to the genetic background of the strains (Fig. 1c).

Interspecific Cross

There was a four-fold difference in the level of sensitivity to fenpropimorph between the *P. anguioides* (ED50 = 6.9 μM) and the R-type *P. herpotrichoides* (ED50 = 28.3 μM) parental strains. Fenpropimorph sensitivity of the interspecific hybrids showed considerable variation, similar to that seen with the WxR cross, suggesting that several genes are involved in resistance expression (Fig. 2a). Both super-sensitive and super-resistant progenies were recovered.

Difenconazole (Fig. 2d) identified a sensitive and resistant subset of progeny of which the difenconazole-sensitive strains were also the most sensitive to fenpropimorph (Fig. 2b). While the triadimenol sensitivity of the hybrids was generally similar to the resistant *P. herpotrichoides* parent (ED50 = 278 μM) (Fig 2c), one strain showed increased triadimenol resistance (ED50 = 976 μM). No cross-resistance was seen between triadimenol and difenconazole (Fig. 2d), in contrast to the interpathotype cross results (cf. Fig. 1d), or between triadimenol and fenpropimorph (Fig. 2c). Evidence was obtained, however, of cross-resistance between fenpropimorph and difenconazole (Fig. 2b).

Clearly the gene(s) having a major effect on sensitivity to difenconazole in this interspecific cross is distinct from that detected in the interpathotype (WxR) cross. In both cases, however, the results demonstrate the involvement of a number of genes conditioning resistance to these sterol-inhibiting fungicides in *Pseudocercosporella*.

The cross-resistance relationships revealed here between the triazole fungicides and the morpholine fungicide fenpropimorph have relevance for the field. The use of these compounds to control other cereal pathogens may result in the selection of particular *Pseudocercosporella* genotypes.

FIGURE LEGENDS

Fig. 1. Fungicide response of *P. herpotrichoides* (WxR) interpathotype progenies

(1a) Fenpropimorph sensitivity of WxR progeny compared with the parental strains (W & R). ED50 values expressed as Log10 μM.

(1b, c, d) Detection of cross-resistance between difenconazole, triadimenol and fenpropimorph through correlation of ED50 (μM) values. Parental strains (W & R) indicated by shaded squares; interpathotype progenies shown as circles and classified as sensitive (●) or resistant (○) to the DMI fungicides.

Fig. 2. Fungicide response of *P anguioides* x *P herpotrichoides* (AxR) interspecific hybrids

(2a) Fenpropimorph sensitivity of hybrids compared with the parental strains (A & R). ED50 values expressed as Log10 μM.

(2b, c, d) Detection of cross-resistance between difenconazole, triadimenol and fenpropimorph through correlation of ED50 (μM) values. Parental strains (A & R) indicated by squares; interspecific hybrids shown as circles.

ACKNOWLEDGEMENTS

The authors thank Ciba Geigy and BASF for providing samples of their fungicides. This work was funded by a grant (No.: P15/550) from the Agricultural and Food Research Council.

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Fig. 1. Fungicide responses of WxR interpathotype progenies

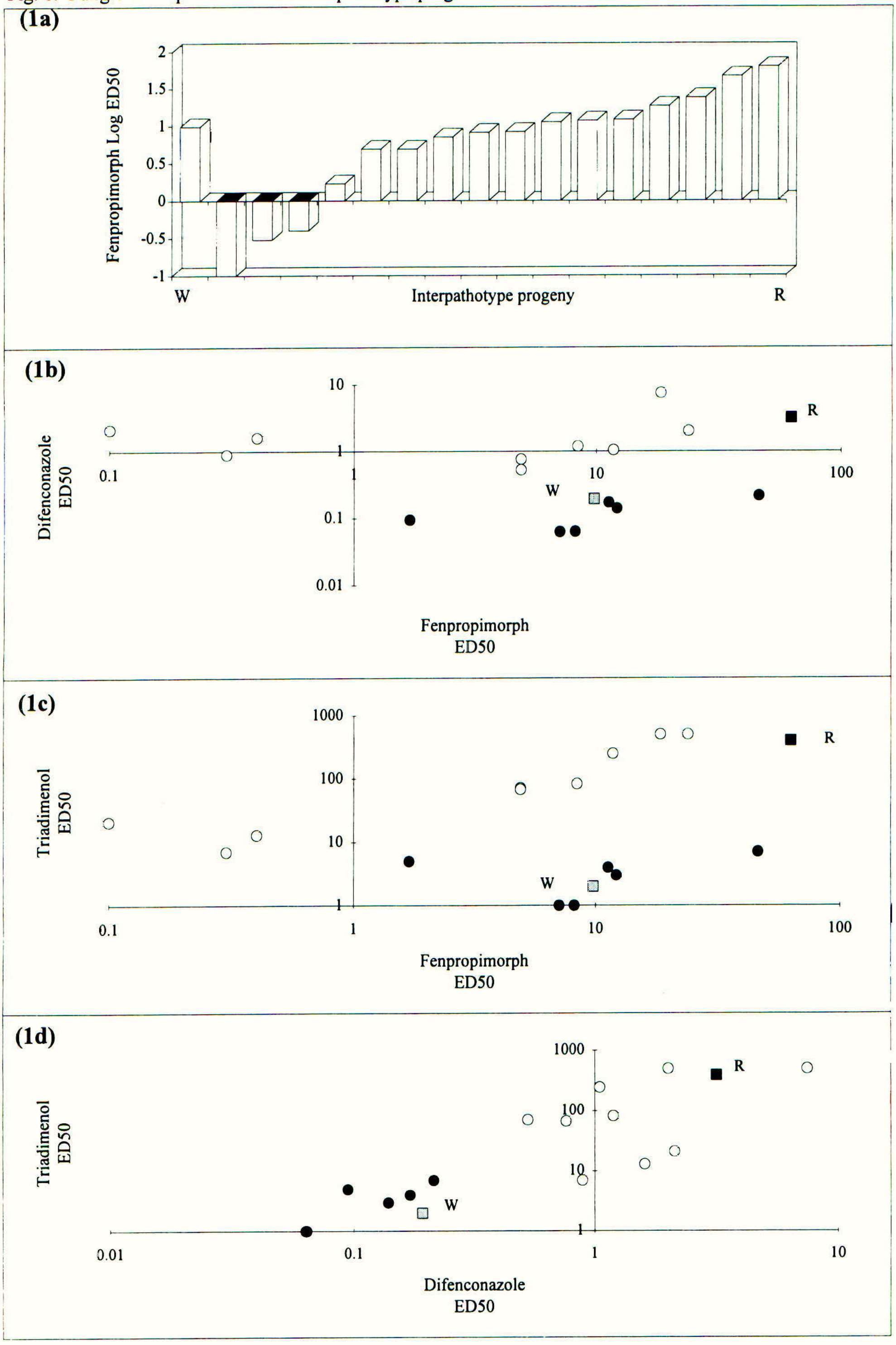
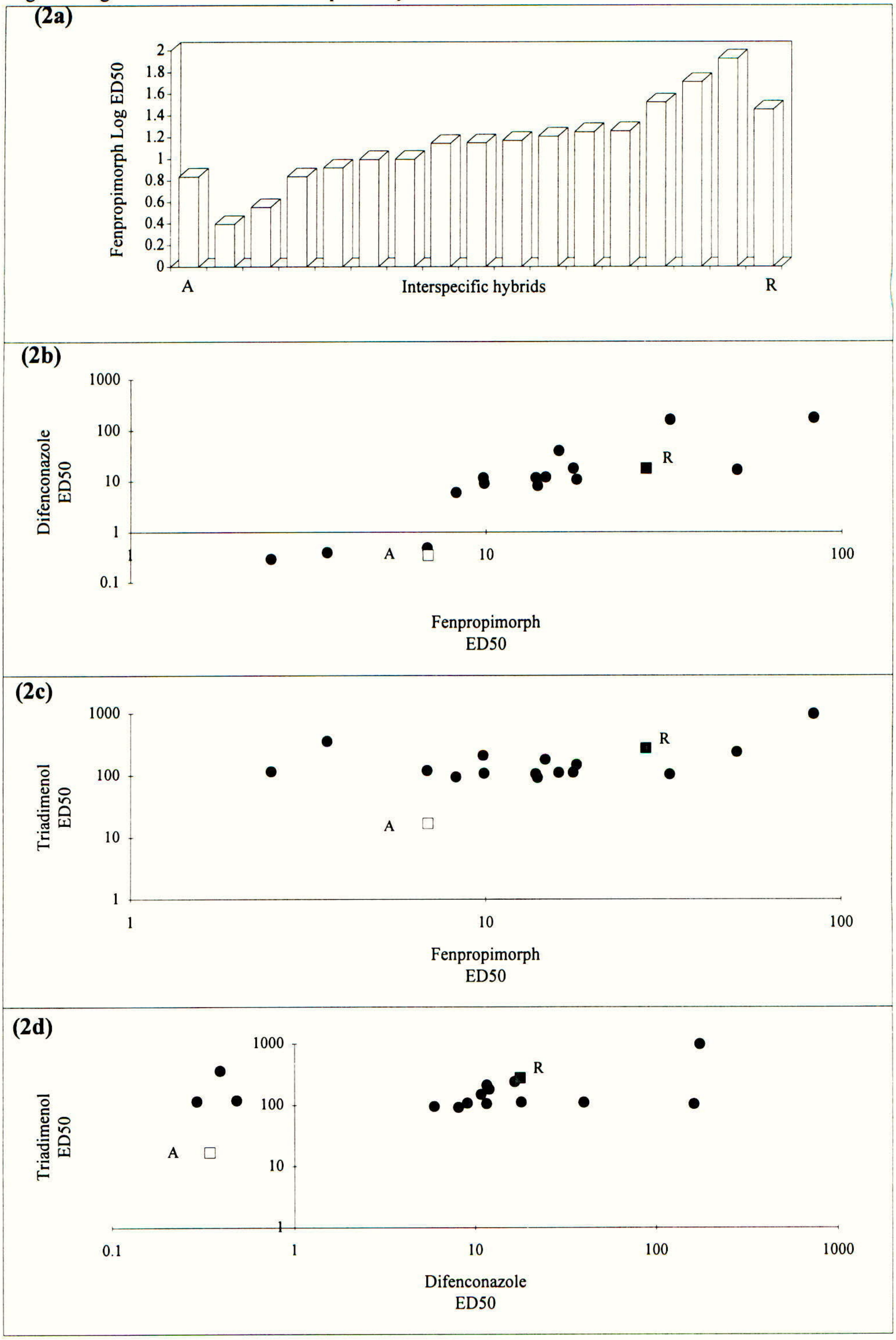


Fig. 2. Fungicide resonses of AxR interspecific hybrids



INFLUENCE OF SPLIT APPLICATION OF FENPROPIMORPH MIXTURES ON DISEASE CONTROL AND ON THE SENSITIVITY OF *ERYSIPHE GRAMINIS* F.SP. *TRITICI*

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ABSTRACT

Mixtures of fenpropimorph with triazoles play an important role in control of *Erysiphe graminis* f.sp. *tritici* and *Erysiphe graminis* f.sp. *hordei*. In Denmark and Northern Germany such mixtures are often used at reduced doses and with more frequent applications than are recommended on product labels. To examine the influence of these split applications on disease control and on the sensitivity to fenpropimorph, field trials were carried out from 1991 to 1993 in Northern Germany. In large plot trials split applications of reduced rates of the fenpropimorph mixture with propiconazole were compared to the recommended two applications of full rates of the mixture and of the solo product fenpropimorph.

Treatments according to the label recommendation provided very good disease control when the timing of applications was adapted to the disease development without producing big changes in fenpropimorph sensitivity at the end of a growing season. Split applications generally resulted in very good powdery mildew control but at the same time showed significant decreases in fenpropimorph sensitivity. Therefore split applications should be avoided.

INTRODUCTION

Mixtures of fenpropimorph with triazoles play an important role in the control of powdery mildew (*Erysiphe graminis* f.sp. *tritici* and *Erysiphe graminis* f.sp. *hordei)* on wheat and barley. Different trials (Bolton and Smith, 1988; Lorenz *et al.*, 1992) have shown the benefit of such combinations for disease control and to combat the development of resistance to either mixture partner. In Denmark and Northern Germany such fenpropimorph mixtures are often used at reduced dose-rates and more frequently than recommended on the label. This may have contributed to a sensitivity shift for fenpropimorph also in this area. Felsenstein (1991) and Lorenz *et al.* (1992) found about a ten-fold decrease of powdery mildew sensitivity to fenpropimorph in this region which occurred already in 1989.

To examine the influence of split applications on the sensitivity to fenpropimorph and at the same time on disease control, specially designed field trials were carried out from 1991 to 1993 in commercial fields in Northern Germany, in an area of high disease pressure for wheat powdery mildew. The influence of split applications of fenpropimorph plus propiconazole was compared to the full rate of fenpropimorph and to the recommended two applications of the mixture.

MATERIALS and METHODS

The field trials were carried out from 1991 to 1993 in Niedersachsen, Germany, on the wheat variety *Kanzler* in different commercial fields. The following treatments were included:

I = check (untreated)
II = fenpropimorph (fpm) at 750 g/ha (recommended rate)
III = fenpropimorph at 300 g/ha in combination with propiconazole (ppz) at 125 g/ha (recommended rate)
IV = fenpropimorph at 150 g/ha in combination with propiconazole at 62.5 g/ha
V = fenpropimorph at 75 g/ha in combination with propiconazole at 31.25 g/ha

TABLE 1. Timing of application related to growth stages (GS) (Zadoks *et al.*, 1974)

Treat-ment	Year	Timing of application (GS)										No. of treatments	% Attack[1] at 1. application
		29	30	31	32	33	34	37	39	49	55		
II	1991			X					X			2	3-4
II	1992			X						X		2	1-3
II	1993				X				X			2	3
III	1991			X					X			2	3-4
III	1992			X						X		2	1-3
III	1993				X				X			2	3
IV	1991			X		X			X	X		4	3-4
IV	1992		X	X				X		X		4	0.5-1
IV	1993			X		X		X	X			4	2
V	1991	X	X	X		X	X		X	X		7	0.5
V	1992		X	X	X			X		X	X	6	0
V	1993			X	X	X		X	X			5	1

[1] percentage powdery mildew attack on green plant tissue

The timing of the first application in the different plots was adapted to the disease development in each year. In treatment V the first application was always carried out when 0-1 % powdery mildew was visible. In 1991 and 1993 the first application in the plot IV was started at the same time as in the full rate plots (II, III) with a disease level of 2-4 %, whereas in 1992 the treatment in this plot was started earlier at 0.5-1 % powdery mildew attack.

The full rate plots (II, III) were treated on average at 24 day intervals; twice per season. The average application interval in the split application plot IV was 10 days and a total of four applications per season were carried out each year. In plot V the spray interval averaged 9 days. In 1991 seven, in 1992 six and in 1993 five applications were carried out in treatment V.

To assess the initial sensitivity to fenpropimorph bulk samples of ten leaves infected with powdery mildew per sample were collected each year before any application was carried out, around growth stage (GS) 29-30 (Zadoks *et al.*, 1974). About 15 days after the last treatment samples were again taken from the different plots. The average number of samples collected in each plot and at the two sampling times was twelve.

Disease rating was carried out by visual assessment of the percentage powdery mildew infection on green plant tissue. In 1991 and 1992 the final rating was carried out at the beginning of July whereas in 1993 this was at the beginning of June.

The average plot size in 1991 was 25000 m^2, 1992 11900 m^2 and in 1993 9900 m^2. However in 1993 the check was integrated as a small untreated part into the middle of a treated area beside the trial plots.

The sensitivity was tested with the test tube method (Sozzi *et al.*, 1991). The concentration range was adapted for fenpropimorph: 0, 0.01, 0.03, 0.1, 0.3 and 1 mg/l AI. EC50-values were determined graphically on a semilogarithmic scale. A sensitive reference isolate (EC50 = 0.03 ppm AI) was included in each test. For a better comparison of results between different tests Q50-values were calculated which represent the ratio of the EC50 value of each tested isolate and the EC50 of the reference isolate in the same test.

RESULTS

Fenpropimorph sensitivity

In table 2 the sensitivity data for the different years are shown as mean Q50-values per treatment. The sensitivity in the different plots did not differ significantly before treatments were carried out. Therefore in the first line the mean Q50-values for all plots are pooled. However the range of Q50-values was broad at the beginning of each tested season: in 1991 Q50-values ranged between 1 and 8, in 1992 between 3 and 9 and in 1993 between 2 and 7.

In 1991 a slight but significant decrease in sensitivity to fenpropimorph was found in all treated plots compared to the check. The most pronounced decrease in sensitivity was detected in the plot with split application IV which differed significantly from the treatment (II) with the solo product fenpropimorph at the full rate. 1992 results show the highest decrease in sensitivity for the split application plot V with 6 treatments. This treatment showed a significant difference to the full rate of the solo product (II) and the mixture (III). Results of 1993 confirm again the highest decrease in sensitivity with the two split applications IV and V. Compared to the previous years, in 1993 the highest Q50-values were found in these plots at the end of the season. Compared to the sensitivity situation early season (reference) the sensitivity decreased by a factor of about two to three. In 1992 and 1993 these changes were more pronounced in treatment V than following fewer applications in treatment IV. In all three years of testing in these plots at the end of the season the very sensitive isolates with Q50-values between 1 and 4 were lost (data not shown).

No significant differences were detected between the application of the solo product and the mixture at the recommended full rate. For both treatments the changes in sensitivity were smaller than for the split applications.

The check-plot in 1993 also shows a higher mean Q50-value at the end of the season. This is probably due to the fact, that the plot size was reduced and that it was not well separated from the trial plots.

TABLE 2. Sensitivity of *Erysiphe graminis* f.sp. *tritici* to fenpropimorph at the beginning and at the end of the growing season in the monitoring trials of the different years.

Treatment	Mean Q50-value for fenpropimorph		
	1991	1992	1993
reference, early season[1)]	3.56	5.31	4.62
I: check[2)]	4.12 a	5.27 a	6.96 a
II: 2 x fpm 750 g/ha[2)]	6.45 b	6.21 a	6.73 a
III: 2 x fpm 300 g/ha + ppz 125 g/ha[2)]	6.91 bc	5.30 a	6.39 a
IV: 4 x fpm 150 g/ha + ppz 62.5 g/ha[2)]	7.83 c	7.31 ab	10.43 b
V: 5 - 7 x fpm 75 g/ha + ppz 31.25 g/ha[2)]	7.08 bc	8.42 b	12.18 b

Means followed by the same letter in the same column do not differ significantly, LSD P = 0.05.
[1)] before first treatment, all plots pooled [2)] after last treatment

Powdery mildew levels

Powdery mildew levels at the beginning of June and July are shown in table 3 for the different treatments, and for the three trials carried out from 1991 to 1993.

In 1991 the best performance through to the end of the season was obtained with two treatments of the full rate of fpm at 750 g/ha and with the mixture of 300 g/ha fpm plus 125 g/ha propiconazole. The highest powdery mildew level was found with four applications of the mixture of fenpropimorph at 150 g/ha plus propiconazole at 62.5 g/ha. Seven applications of the mixture fenpropimorph at 75 g/ha plus propiconazole at 31.25 g/ha (V) showed very good disease control (about 0.5 % attacked green plant tissue) at the beginning of June. But at the end of the growing season the powdery mildew level increased to 5 % attacked green plant tissue. However generally all treatments performed well.

In 1992 the best performance was obtained with the two split applications (IV, V). Six applications of fenpropimorph at 75 g/ha in combination with propiconazole at 31.25 g/ha (V) even showed better powdery mildew control than four applications of fenpropimorph at 150 g/ha in combination with propiconazole at 62.5 g/ha (IV). However 1992 was an exceptional year due to a severe drought during May with very low disease pressure. The first application in the full rate plots (II, III) was carried out mid May at 1-3 % powdery mildew attack and in the plot IV in the first week of May with an infection level of *Erysiphe graminis* f.sp. *tritici* of 0.5-1 %. The first rainfalls were recorded end of May and the powdery mildew attack in the check plot immediately reached 10 %. The second application of the full rate (II, III) was carried out 1 week later and so the timing of application in these plots was not related to the powdery mildew development.

Performance in 1993 was good in all treated plots, especially in those receiving fenpropimorph alone (II) or split applications (IV, V). However no late disease rating was carried out and therefore no clear conclusions on performance of the different treatments can be drawn.

TABLE 3. Powdery mildew levels on wheat at two dates during three seasons in full dose and split application treatments.

Treatment	% attacked green tissue					
	1991		1992		1993	
	June[1]	July[2]	June	July	June	July
I: check	20	30	19	45	50	nd
II: 2 x fpm 750 g/ha	1	1	3	20	2	nd
III: 2 x fpm 300 g/ha + ppz 125 g/ha	1-2	1-2	7	25	7	nd
IV: 4 x fpm 150 g/ha + ppz 62.5 g/ha	5	7	2	10	2	nd
V: 5 - 7 x fpm 75 g/ha + ppz 31.25 g/ha	1	5	1	5	1	nd

nd: not determined

[1] beginning of June [2] beginning of July

DISCUSSION

As a rule split applications resulted in very good powdery mildew control in all three trial seasons. In these treatments the disease rating at the end of the growing season showed an increase in powdery mildew level compared to the normal applications only in 1991.

With the treatments according to the label recommendation (two applications at the full rate) performance was also very good when the timing of application was adapted to the disease development. The importance of application timing is also shown for the split applications. More frequent treatments generally performed better than fewer treatments. Similar results were obtained by Jørgensen and Nielsen (1992) for *Erysiphe graminis*, *Puccinia striiformis* and *Septoria* spp. on wheat in Denmark.

The three trials reported here show that with the more frequent application at the reduced dose (split application) bigger shifts towards decreased sensitivity occur than with two applications of the full rate. This is a consequence of a continuous selection pressure resulting in a higher frequency of isolates with reduced sensitivity to fenpropimorph at the end of a season. With two treatments, of either fenpropimorph alone or the mixture with propiconazole, at the recommended rate and timing, changes in sensitivity were smaller and at the same time a good performance was obtained.

These results suggest that the application of the recommended label rates at the right time represent the optimum between the prevention of sensitivity shifts and excellent powdery mildew control. With split applications the margin of error in timing of applications decreases but clearly the risk for sensitivity shifts becomes much greater. Similar results were obtained in monitoring trials carried out in The Netherlands by T. Engels (personal communication, 1994). Thus it appears that the best application would be two treatments of the full rate at the best possible timing. This recommendation was also adapted by the FRAC-DMI working group (1993).

It remains to be determined whether the results shown here may also be extrapolated to other pathosystems.

ACKNOWLEDGEMENTS

The authors would like to thank Mr. H. W. Boedeker for carrying out the field trials and Mrs. O. Schafer for skilled assistance in carrying out the sensitivity tests.

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RECENT EVOLUTION AND CURRENT STATUS OF SENSITIVITY OF ERYSIPHE GRAMINIS F.SP. TRITICI TO FENPROPIMORPH IN DIFFERENT EUROPEAN REGIONS

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ABSTRACT

To study sensitivity and population dynamics of the wheat mildew pathogen towards fenpropimorph on an European scale, a monitoring program has been carried out since 1987. Single isolates out of different regions were analysed. In relation to standard isolates with unselected sensitivity a resistance factor and for samples their mean (MRF) were calculated. The current results show regional differences in sensitivity of the pathogen towards fenpropimorph. In the east and south of Europe populations remained on their former sensitivity level whereas most populations in north-western Europe changed recognizably to a MRF $\geq$ 3 within recent years, due to selection pressure and wind dissemination of the pathogen. Compared with resistance evolution to azoles, sensitivity adaptation towards fenpropimorph appears to progress more slowly. Until 1993 only few populations reached MRFs of about 10. At the same time this sensitivity level seems to be like a first limit for resistance evolution. The latter is discussed to be due to the fact that morpholines are two-site-inhibitors and to the genetical recombination of the wheat mildew pathogen.

INTRODUCTION

Controlling wheat powdery mildew, caused by Erysiphe graminis f.sp. tritici, treatments are up to the present mainly based on two groups of active compounds, namely azoles (DMIs: demethylation inhibitors) and morpholines/morpholine-like-compounds, which have different modes of action inhibiting sterol biosynthesis of the fungus. Whereas during the last decade a partly evident sensitivity loss of the pathogen towards DMIs was monitored mainly for the north-west of Europe (Felsenstein, 1994), and a lot of data are available, there is a lack of information about sensitivity of the wheat powdery mildew towards morpholines on an European scale. This report provides a short survey on the recent evolution and current status of fenpropimorph sensitivity of the wheat mildew pathogen in Europe.

MATERIALS AND METHODS

Random samples of the pathogen were taken out of the air above regions of interest making use of a mobile jet spore trap. Single progenies of collected conidia spores were analysed for their sensitivity towards fenpropimorph. ED50 was calculated for each isolate tested. In relation to the mean ED50 of unselected standard-isolates a resistance factor was determined. To characterize each random sample the mean ED50 (geometric mean) and the mean resistance factor **MRF** is used (more details in Felsenstein, 1991; Felsenstein, 1994).

RESULTS

Current data about fenpropimorph sensitivity of random samples of wheat mildew out of different European regions are presented in Table 1. Region (route) and date of spore sampling, number of isolates tested, mean of ED50 values of single isolates within the random sample, its standard deviations and the total range are listed. For standard isolates the method used at Weihenstephan resulted ED50 values around 0.15 ppm. The mean ED50 of the samples ranged from 0.24 ppm in northern Italy up to 1.98 ppm in Scotland. Thus, at the current status of sensitivity there exists a visible regional differentiation towards fenpropimorph on an European scale. Sensitivity levels close to that of the standard isolates were observed for populations in southern and eastern Europe. Recognizable lower levels were found in general for populations in the north-west of Europe. But despite of a changed sensitivity level isolates with ED50 values close to those of the standards were still obtained in most samples.

A different situation was observed at the beginning of monitoring in 1984 (Lorenz & Pommer, 1984) and at the start of our investigations in 1987. In Table 2 mean resistance factors (MRFs) of the samples out of the wheat mildew populationes listed in Table 1 are presented for a seven year period from 1987 to 1993. In 1987 only two populations, one in Scotland ('Edinburgh-Grantshouse') and one in northern Germany ('Hamburg-Neustadt'), had a markably higher MRF value, if populations with MRF $<$ 3 are considered to possess an unselected or nearly unchanged sensitivity level (Felsenstein, 1991). In the following years more and more populations of north-western Europe attained a level of MRF $\geq$ 3. As mentioned above, only populations in the south and east of Europe remained on their former

TABLE 1. Sensitivity of wheat powdery mildew from different European regions to fenpropimorph, 1993

region	date	n	$\bar{x}$ED50*	$\bar{x}$-s - $\bar{x}$+s	min. - max.
GB:					
Edinburgh-Grantsh.	05.07.	10	**1.98**	1.50 - 2.62	1.43 - 3.15
Cambridge-Dover	05.07.	10	**1.74**	1.17 - 2.60	0.61 - 2.44
F:					
Calais-Lille	04.07.	10	**1.13**	0.65 - 1.98	0.24 - 1.63
Paris-Reims	18.06.	10	**0.81**	0.40 - 1.65	0.22 - 1.53
Bourges-Nevers	19.06.	10	**0.97**	0.37 - 2.57	0.18 - 2.37
Auch-Toulouse	26.05.	10	**0.43**	0.26 - 0.71	0.21 - 1.15
DK:					
Nyborg-Kopenhagen	15.06.	20	**1.36**	0.78 - 2.38	0.22 - 2.63
D:					
Hamburg-Neustadt	16.06.	18	**1.46**	0.85 - 2.52	0.23 - 2.63
Hannover-Kassel	12.06.	10	**1.23**	0.89 - 1.71	0.55 - 1.72
Dortmund-Warburg	15.06.	10	**0.75**	0.26 - 2.18	0.16 - 4.60
Magdeburg-Halle	12.06.	10	**0.86**	0.40 - 1.87	0.21 - 1.61
Nürnberg-Freising	05.06.	10	**0.57**	0.21 - 1.57	0.12 - 1.77
Pforzheim-Ulm	04.06.	10	**0.60**	0.30 - 1.22	0.20 - 1.43
CH:					
Baden-Bern	17.07.	10	**0.78**	0.37 - 1.64	0.28 - 1.86
A:					
Marchfeld/by Vienna	11.06.	10	**0.35**	0.19 - 0.62	0.20 - 1.43
I:					
Verona-Venedig	25.05.	10	**0.24**	0.21 - 0.28	0.20 - 0.31
standard isolates:					
"Benno"		5 x	**0.17**	0.15 - 0.20	0.15 - 0.21
"Sappo"		7 x	**0.16**	0.15 - 0.17	0.15 - 0.17
"W72"		4 x	**0.15**	0.12 - 0.19	0.11 - 0.19

* geometric mean of ED50 values of single isolates in the sample; concentration of the active component in mg/l (ppm)

TABLE 2. Mean resistance factors (MRFs) of random samples out of regional wheat mildew populations towards fenpropimorph in Europe, 1987-1993

region	1987	1988	1989	1990	1991	1992	1993
GB:							
Edinburgh-Grantsh.	4.2	1.7	3.1	4.9	4.2	5.6	12.4
Cambridge-Dover	1.8	1.3	2.0	4.0	2.6	7.0	10.9
F:							
Calais-Mons/Lille	1.1	1.8	2.0	2.3	4.8	1.6	7.1
Paris-Reims	1.3	2.6	2.1	1.8	2.2	11.9	5.1
Bourges-Nevers	-	-	-	1.4	1.3	4.0	6.1
Narb./Auch-Toulouse	2.1	-	1.7	-	1.4	1.3	2.7
DK:							
Nyborg-Kopenhagen	2.3	3.5	10.3	7.1	10.5	8.9	8.5
D:							
Hamburg-Neustadt	7.5	4.9	9.0	9.2	10.6	10.5	9.1
Hannover-Kassel	-	-	-	8.3	8.1	6.4	7.7
Dortmund-Warburg	2.1	1.9	11.8	-	10.9	7.3	4.7
Magdeburg-Halle	-	1.4	2.8	3.0	6.8	5.7	5.4
Nürnberg-Freising	2.7	1.5	2.0	1.6	2.9	3.6	3.6
Pforzheim-Ulm	2.3	1.4	1.7	1.6	1.9	5.6	3.8
CH:							
Baden-Bern	-	1.2	1.9	1.5	1.5	2.0	4.9
A:							
Marchfeld/by Vienna	1.4	2.4	2.6	1.7	1.6	1.4	2.2
I:							
Verona-Venedig	-	1.1	1.6	1.3	1.1	1.2	1.5
E:							
Irun-Tudela	0.9	-	1.2	-	0.8	1.2	-

level close to that of the standard isolates.

At the same time it has to be stressed that up to 1993 **no** population could be detected which reached a markably higher MRF than 10. This fact also holds true for the population of the region 'Hamburg-Neustadt' where MRF varied between ca. 5 and 10 during the whole investigation period.

DISCUSSION

Results presented show that the wheat mildew pathogen possesses the ability in sensitivity adaptation towards the morpholine compound fenpropimorph on principle. As MRF values > 3 have been obtained from areas with a relative frequent (e.g. splitting) and extensive use of appropriate fungicides, respectively, it is considered that the loss of sensitivity mainly depends on selection pressure. In the recent past also wind dissemination of the pathogen out of regions with higher MRFs seems to have an increasing influence on the sensitivity of some regional populations. For few populations (e.g. 'Edinburgh-

Grantshouse', 'Dortmund-Warburg') an evident variation in MRF-value from year to year has been obtained. This observation is characteristic for populations which got a wide range within their sensitivity structure.

At the same time results show a relatively moderate population dynamic according to a loss of sensitivity to fenpropimorph during the last decade. In most populations of north-western Europe sensitivity level changed markably at first at the beginning of the 90s. Only population structure did indicate changes at an earlier time (Felsenstein, 1991; Lorenz *et al.*, 1992). The main reason for the moderate resistance evolution and the slightly reduced sensitivities to fenpropimorph might be due to the fact that morpholines are considered to be a two-site-inhibitor (Buchenauer, 1984; Kato, 1986; Kerkenaar, 1987). In addition the polygenic control of resistance evolution towards morpholines combined with a markably genetical recombination of the wheat powdery mildew should be taken into account, as it is discussed for sensitivity of the pathogen to DMIs (Felsenstein, 1994). Because of the yearly redistribution of genes responsible for a loss in fenpropimorph sensitivity, maintenance and multiplication of pathotypes with higher resistance factors are probably hampered.

Nevertheless it is astonishing that despite of a high selection pressure during the last decade sensitivity level of the population in the region 'Hamburg-Neustadt' has **not** changed markably since at least 1987. This observation as well as data from other populations indicate that a MRF of about 10 is a first serious limit towards a furthermore adaptation in fenpropimorph sensitivity of the wheat mildew pathogen. Therefore successful pathogen control seems likely to continue at least in the near future.

ACKNOWLEDGEMENT

The author wishes to thank Maria Goldbrunner, Mariele Felsenstein, Johanna Stieber and Hannelore Meckl for excellent technical assistance.

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EFFECT OF DIFFERENT TREATMENT SCHEDULES ON THE SENSITIVITY OF *ERYSIPHE GRAMINIS* F.SP. *TRITICI* TO FENPROPIMORPH

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ABSTRACT

The effects of Corbel and Tilt Top split applications on disease control, and on sensitivity in *Erysiphe graminis* f.sp. *tritici* to fenpropimorph, were tested in field experiments in 1992 and 1993. In both years, wheat powdery mildew was controlled well in all treated plots. In general, the use of split applications resulted in a better protection against wheat powdery mildew than the use of normal applications. In the 1992 experiment no differences in sensitivity were found between split and normal applications. In the 1993 experiment however, isolates collected from plots treated with split applications had on average the highest EC_{50}- and Q_{50}-values (degree of resistance). This suggests that split application favours reduction in sensitivity to fenpropimorph.

Competitive ability of isolates with a reduced sensitivity to fenpropimorph was studied. Isolates with a reduced sensitivity showed a lower competitive ability than wild type isolates.

INTRODUCTION

One of the main problems in chemical control of plant diseases is resistance development in target organisms. This risk also applies to the fungicide fenpropimorph in the control of *Erysiphe graminis* f.sp. *tritici*, the causal agent of wheat powdery mildew. Some decrease in the sensitivity of this pathogen to fenpropimorph has already been found in a number of West-European countries (De Waard et al., 1992). As far as treatment schedules are concerned, the use of the single product, and the use of a mixture with a companion fungicide both in full rates as well as in split applications of the recommended rates may influence the build-up of strains with decreased sensitivity either positively or negatively.

Resistance development is influenced by the competitive ability of isolates with reduced sensitivity. When competitive ability of less sensitive isolates is lower than those of wild-type isolates, isolates with reduced sensitivity will only develop when a continuous selection pressure of the fungicide is present.

AIM OF THE RESEARCH

I. To study the effect of split application of fenpropimorph, as a single product and as a mixture, on the sensitivity of wheat powdery mildew.
II. To investigate the competitive ability of fenpropimorph-sensitive (FS) and -low resistant (FR) wheat powdery mildew isolates.

MATERIALS AND METHODS

I. Field trials were conducted in the Southern Netherlands in 1992 (pilot experiment) and 1993. Two fungicides, "Corbel" (a.i. fenpropimorph 750 g/l) and "Tilt Top" (a.i. fenpropimorph, 375 g/l and propiconazole 125 g/l), were applied as split (5 x 0.4 l/ha) or normal (2 x 1.0 l/ha) applications. The experimental field of 1993 was situated next to the one used in 1992. Powdery mildew development was assessed in all plots during the growing season. Plots were sampled for plants with mildew symptoms before the start of treatments and after all treatments were applied. Isolates collected were used in foliar spray tests to assess the sensitivity to fenpropimorph. Foliar spray tests were carried out in a spray cabinet in a standardized way. After spraying, the seedlings were inoculated and after 10-12 days of incubation, mildew severity of the seedlings was estimated. EC_{50}- and Q_{50}-values were calculated. The degree of resistance (Q_{50}-value) was calculated by dividing the EC_{50}-value of a field isolate by the average EC_{50}-value of a reference isolate LH (wild type) tested in the same test.

II. To study the competitive ability of FS- and FR- monospore isolates, two FS-isolates (LH and 67) and two FR-isolates (3a and 16c) were mixed on eight day old wheat seedlings (cv Okapi) in 12.5 cm diameter pots. In this way four mixtures, which consisted of four isolates, were made. These four mixtures and the separate FS- and FR-monospore isolates were transferred every week to unsprayed wheat seedlings (cv Okapi). These competition experiments were carried out using inoculum levels that assured 90% infection. The composition of the mixtures was assessed by transfer of 50 colonies to wheat seedlings sprayed with a discriminating concentration (11 $\mu g\ ml^{-1}$) of fenpropimorph. In contrast to FS-colonies, FR-colonies survived this treatment. Tests were done at the start of the experiment (week 0) and 1, 3, 6, 10 and 15 weeks after the start of the experiment.

RESULTS

I. Powdery mildew development

In both years, only small differences in mildew infestation were found between plots at the start of the treatments (DC31). During the course of the season leaves in the untreated plots got more infected than leaves of the other plots. Wheat powdery mildew was controlled well in all treated plots in both years. In general, split application Corbel and Tilt Top resulted in less powdery mildew development than normal application of these fungicides (Figure 1).

Sensitivity to fenpropimorph

The mildew population in the experimental field selected had a decreased sensitivity to fenpropimorph. At the start of the treatments in both years no significant differences in sensitivity were found between isolates from different plots. At the end of the 1992 pilot experiment, the sensitivity of isolates collected from plots with normal and split applications was similar in all treatments (data not shown). At the end of the 1993 experiment, the degree of reduced sensitivity of isolates collected from plots treated with split applications was higher than for isolates from all other plots (Table 1).

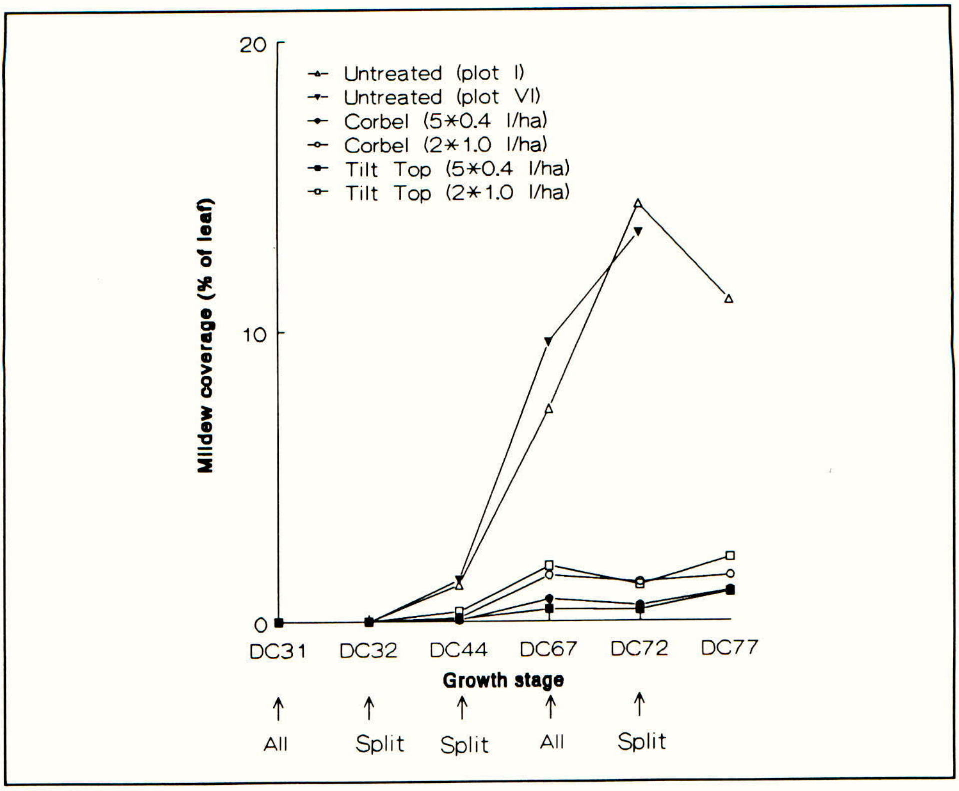

Figure 1. Development of *Erysiphe graminis* f.sp. *tritici* on the second leaf of wheat in six plots during the growing season of the 1993 field trial. Split: fungicide application in split application plots only. All: fungicide application in all plots.

Table 1. Average EC_{50}-values (μg fenpropimorph ml^{-1}) and Q_{50}-values of fenpropimorph for powdery mildew control in foliar spray tests with isolates from six plots in the 1993 field trial, I and VI = Untreated, II = Corbel 5 x 0.4 l/ha, III = Corbel 2 x 1.0 l/ha, IV = Tilt Top 2 x 1.0 l/ha and V = Tilt Top 5 x 0.4 l/ha.

	Treatment						LSD
	I	II	III	IV	V	VI	
EC_{50}	7.9 a	13.8 c	10.0 b	9.8 b	12.6 c	6.6 a	1.7
Q_{50}	6.2 a	11.0 c	8.0 b	7.8 b	10.0 c	5.3 a	1.3

Means followed by the same letter in the same row do not differ significantly, P = 0.05.
Total amount (g) of a.i. of fenpropimorph: I and VI: 0, II and III: 1500, IV and V: 750.

II. Competitive ability assessments

Mixtures consisted of 55% FR-isolates. The frequency of FR-isolates in the mixtures gradually decreased during the course of the experiment reaching 15-20% after 15 generations (Figure 2). The sensitivity of the FS- and FR- monospore isolates did not change during the course of the experiment.

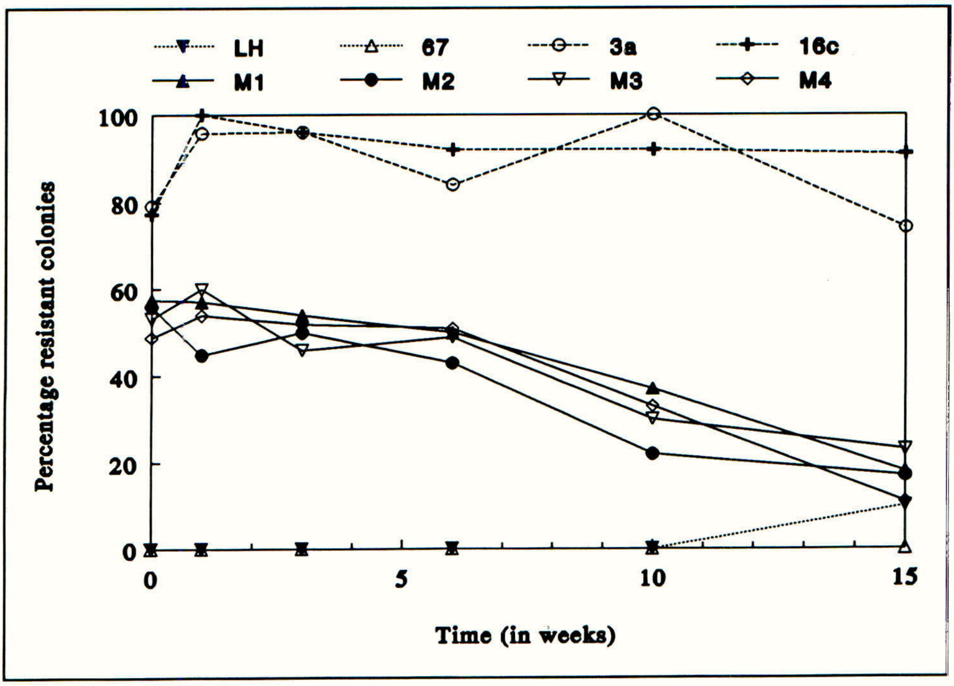

Figure 2. Changes in the composition of mixtures (M1...M4) and monospore isolates (FS: LH and 67; FR: 3a and 16c) at 20°C in the absence of fenpropimorph.

DISCUSSION

I. Powdery mildew development was highest in the untreated plots. In all treated plots mildew was controlled well. In general, split applications performed better in mildew control than normal applications. Similar results were obtained by Jørgensen and Nielsen (1992). Probably, split application results in a better timing of the application in relation to leaf emergence and hence more effectively prevents mildew development. No difference in mildew development between applications of Corbel and Tilt Top were observed. These results were similar in both years.

In both years, isolates collected before the start of the treatments showed no significant difference in sensitivity to fenpropimorph. In the 1992 experiment no differences in sensitivity were found in isolates from plots treated with split and normal applications. In the 1993 experiment, results of foliar spray tests demonstrated that isolates from plots treated with split application had on average the highest EC_{50}- and Q_{50}-values and isolates collected from untreated plots the lowest. This result suggests that in

1993 split applications favour stepwise reduction in sensitivity to fenpropimorph. Forster *et al.* (1994) found similar results in monitoring trials carried out in Northern Germany. A reason for the difference in results of 1992 and 1993 may be that in the 1993 experiment disease pressure was much higher than in the 1992 experiment. Also the fact that 1993 was the second successive year with a high selection pressure may have contributed to the 1993 results.

II. The results show that under crowded conditions the competitive ability of FR-isolates was less than that of FS-isolates. This suggests that in periods without fenpropimorph application and a high mildew infection the frequency of FR-isolates in practice may decrease.

ACKNOWLEDGEMENT

The authors would like to thank Ir. B.C. Mantel for his excellent contribution to the research. This project is fully supported by CIBA-GEIGY AG, Basel and BASF AG, Limburgerhof.

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