SESSION 6

PESTICIDE RESISTANCE: PREDICTION AND OCCURRENCE

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POSTER PAPERS 6A-1 to 6A-20
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FUNGICIDE RESISTANCE ON TOMATOES IN ITALIAN GREENHOUSES [1] [2]

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ABSTRACT

Resistance of Botrytis cinerea to benzimidazoles and dicarboximides was monitored on tomatoes in several greenhouses of the Albenga area (Northern Italy). Resistance to benzimidazoles was present in 81 to 94 percent of the tomatoes tested. In the case of dicarboximides, resistance has increased from 25% in 1982 to more than 60% in 1983-84. In spite of this increased presence of dicarboximide resistant strains, vinclozolin still provides the best disease control.

INTRODUCTION

Gray mould (Botrytis cinerea) can seriously damage a range of vegetable crops in Italy. Although several fungicides are available for use against the pathogen, only two groups of compounds, benzimidazoles and dicarboximides, can completely control the fungus. Unfortunately the presence of benzimidazole-resistant strains in the population of the pathogen has limited the use of benzimidazole compounds in recent years. Other fungicides such as captan, captafol, folpet, chlorthalonil and dichlofluanid provide only partial control, especially when climatic conditions are favourable for the development of gray mould. Since their appearance on the market (1979), dicarboximides have been widely used on several crops, and have given perfect control of B. cinerea. In some greenhouses, they have been the only fungicides sprayed against gray mould and often have been used on successive crops. In 1981 severe attacks of gray mould were observed in two tomato greenhouses near Albenga in Northern Italy in spite of regular applications of dicarboximides. From 90% of the infected fruit tested, strains of B. cinerea resistant to dicarboximides were isolated. The majority of these strains were also resistant to benzimidazoles (Gullino et al., 1981). In both greenhouses, tomatoes had been grown after basil and the two crops had been sprayed with dicarboximides against gray mould 10-11 times during a period of 5-6 months.

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At the same time in other greenhouses in the same area where dicarboximides had been used less frequently (3-4 times/season), no failure in disease control was observed. From the end of 1981, an increasing number of growers reported reduced effectiveness of dicarboximides. Decreased activity of dicarboximides on vegetable crops, due to the presence of resistance, has also been reported in Greece and Israel (Panagiotaku & Malathrakis 1981, Katan 1982).

A monitoring programme was started in several greenhouses of the Albenga area in order to detect the presence of dicarboximide-resistant strains and to evaluate their incidence in the pathogen population. At the same time, resistance to benzimidazoles was also monitored: the presence of benzimidazole-resistant strains on tomato in Italy is well known, but there is no information concerning its frequency.

MATERIALS AND METHODS

The monitoring was carried out during the years 1982 to 1984 in the horticultural area of Albenga. Isolations were made from sporulating lesions by means of a collecting spore device used in the greenhouses when possible, or on samples sent to the laboratory. If the lesions were not sporulating, samples were kept for 24-48 hours in high humidity conditions to induce sporulation. Plates contained PDA amended with terramycin (50 µg/ml) and zineb (20 µg/ml) in order to avoid the development of contaminants. Resistance to dicarboximides was monitored by using 3 µg/ml of vinclozolin; in the case of benzimidazoles, 10 µg/ml of benomyl were added to the media. The plates were incubated at 25°C for 3-4 days and observed for the presence or absence of colonies.

When possible, the history of the treatments carried out against gray mould in each greenhouse during the season was recorded.

During 1984 in two greenhouses the effectiveness of different spray programmes on natural mixed populations of strains of B. cinerea sensitive and resistant to dicarboximides was evaluated. The trials were carried out on tomato plants (Lycopersicon esculentum L., cv. 'Nancy') divided into 15 plots. The treatments were carried out every 15 days, starting from flowering, with the fungicides at the dosage reported in table 3. Disease incidence was evaluated by counting the number of infected fruits at the different harvest times. Isolations were carried out as described above, from infected fruits, on PDA containing no fungicide (control) or vinclozolin (3 µg/ml) in order to determine if the rot was caused by sensitive or resis-

tant strains of B. cinerea.

RESULTS AND DISCUSSION

Resistance to benzimidazoles was present in 81 to 94 percent of the tomatoes tested from most greenhouses monitored in 1982, 1983 and 1984 (table 1). In the case of dicarboximides in 1982, resistance was detectable in about 25% of the monitored crops. By 1983, 24 out of 33 greenhouses had resistant strains and 68% of the tested fruits carried resistant conidia. During 1984 resistant strains were recovered in 17 out of 18 greenhouses and all the tested fruits carried resistant conidia in 14 greenhouses (table 1).

It is difficult to find a correlation between the fungicides sprayed and the incidence of resistance to dicarboximides. Growers used from 2 to 15 applications in 5 to 6 months on tomatoes against gray mould and different fungicides (dichlofluanid, captafol+folpet, benzimidazoles, chlorthalonil) were often applied in alternation with dicarboximides. In one greenhouse where dicarboximides were not used during the 1984 season (dichlofluanid was sprayed twice), 100% of the tested fruits showed the presence of dicarboximide-resistant conidia. On the other hand, in the only greenhouse where resistance was not detected, dicarboximides were sprayed three times during the season, in alternation with three treatments with dichlofluanid.

These observations suggest that when dicarboximide-resistant strains are present in a high percentage, it is not possible to eliminate them by the use of other chemicals which are only partially active against B. cinerea.

The results obtained in two tomato trials with natural mixed populations of the pathogen show that, in spite of the large presence of dicarboximide resistant strains, the dicarboximide vinclozolin provides the best disease control. It appears that this includes partial control of resistant strains. Chlorthalonil alone was less effective (table 2). These results agree with those obtained previously with greenhouse grown tomatoes artificially inoculated with mixed populations of sensitive and resistant conidia of B. cinerea and treated with different spray programmes (Gullino et al. 1983).

The present situation of resistance to dicarboximides on greenhouse tomatoes in Italy is certainly serious: the strong selection pressure exerted by frequent and continuous use of dicarboximides selects resistant strains thar survive and spread under favourable greenhouse conditions. Resistance to benzimidazoles and low efficacy by other fungicides like dichlofluanid

and chlorthalonil prevent alternative fungicides being used for the control of gray mould, when dicarboximide resistance is present.

TABLE 1

Resistance to benzimidazole and dicarboximide fungicides in populations of B. cinerea on tomatoes in greenhouse (1983 and 1984)

	Benzimidazoles		Dicarboximides	
	1983	1984	1983	1984
Number of greenhouses monitored	33 (100%)	18 (100%)	33 (100%)	18 (100%)
Number of greenhouses where resistance was detected	30 (91%)	18 (100%)	24 (72%)	17 (94%)
Number of fruit tested	199 (100%)	181 (100%)	199 (100%)	181 (100%)
Number of fruit carrying B. cinerea resistant conidia	163 (81%)	170 (94%)	136 (68%)	133 (62%)
Number of greenhouses where all the tested fruit carried resistant conidia	16 (48%)	15 (83%)	14 (42%)	14 (77%)

Where resistance to dicarboximides is not yet present, a reduced use of these compounds is recommended. For example, other fungicides should be applied at the beginning of the season with the aim of keeping a low level of inoculum and dicarboximides should be sprayed only once or twice at the end of the treatment schedule. Where resistance is present, it may be at a low level which may be controlled to some degree by dicarboximide treatments: therefore, in an emergency, a grower may obtain improved disease control by adding dicarboximide to 1-2 applications of a regular spray programme.

TABLE 2

Effectiveness of different spray programmes on Botrytis rot of tomato fruit and on the presence of B. cinerea dicarboximide--resistant strains

Fungicide	Dosage (g/hl a.i.)	% of rotted fruit		% of rotted fruit carrying dicarboximide--resistant conidia	
		Trial I	Trial II	Trial I	Trial II
Vinclozolin	50	6.33 a[1]	5.33 a	58.8	70.0
Vinclozolin + Chlorthalonil	35 + 75	6.25 a	8.00 a	73.3	85.7
Chlorthalonil	100	18.33 b	25.33 b	64.9	68.0
Control	--	23.33 b	32.66 c	57.1	74.4

[1]Values of each column followed by the same letter are not significantly different, according to Duncan's multiple range test (P = 0.05).

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STUDIES OF A DICARBOXIMIDE RESISTANT HETEROKARYON ON *Botrytis cinerea*

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ABSTRACT

Heterokaryons of *Botrytis cinerea* containing mutant (dicarboximide-resistant) and wild type (dicarboximide-sensitive) nuclei were monitored to determine the changes in the relative proportions of mutant : wild type nuclei during mycelial growth in the presence and absence of fungicide. Results of *in vitro* and *in vivo* experiments indicated that heterokaryosis provides a mechanism for maintaining nuclei carrying mutant genes for dicarboximide-resistance in *B.cinerea* mycelia, even when resistance is associated with low fitness.

MATERIALS AND METHODS

Isolates

Heterokaryotic cells contain two or more genetically different kinds of nuclei in a common cytoplasm, whereas homokaryotic cells contain genetically identical nuclei (Parmeter et al 1963). A dicarboximide resistant heterokaryon (HET), isolated from an iprodione-treated strawberry plot at the research station of May and Baker Ltd. in Essex, contained mutant (dicarboximide-resistant) and wild type (dicarboximide-sensitive) nuclei. Homokaryons were derived from HET by selecting monoconidial progeny on synthetic media. A homokaryotic wild type isolate (HOM-S) was selected on minimal medium, and a homokaryotic mutant isolate (HOM-R) was selected on minimal medium amended with 25µg vinclozolin ml^{-1}.

Media

The isolates were grown on synthetic minimal medium MM, enriched medium CM, and sorbose medium MS (as described by Grindle 1984). Isolates were tested for fungicide resistance on MM containing iprodione (Rovral 50% w.p.) and vinclozolin (Ronilan 50% w.p.).

In vitro experiments

Isolates were compared for differences in growth rate and fungicide resistance (as described by Grindle 1984). Levels of fungicide resistance are expressed as ED_{50} values (µg fungicide ml^{-1} MM that reduces radial growth by 50%). Conidiation was determined by suspending the conidia produced by 11-day-old colonies growing on MM in Tween 80 solution (0.1 ml l^{-1}) and counting with a haemocytometer.

Heterokaryons were incubated at 25°C on dishes (85mm diam) and in special 500mm growth tubes (Ogden and Grindle 1983) containing MM and MM amended with 2.5µg vinclozolin ml^{-1}. The tubes and dishes were inoculated with 8mm^3 blocks of mycelia (HET-S1 and HET-R1). HET-S1 mycelia were obtained after 10 serial subcultures of HET on MM; approximately 0.5% of the monoconidial progeny were dicarboximide-resistant. HET-R1 mycelia were obtained by incubating HET for 5 days on dishes of MM amended with 25µg vinclozolin ml^{-1}; approximately 98% of the monoconidial progeny were dicarboximide-resistant.

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Conidia were sampled from the heterokaryons at intervals during their growth across the dishes and along the tubes, and analysed as described below to determine the proportions of dicarboximide-resistant : dicarboximide-sensitive progeny. The sampling points were 10mm apart in the dishes and 110mm apart in the tubes.

In vivo experiments

The isolates were compared for pathogenicity on tomato leaves. Attempts to infect the leaves with plugs of mycelia were often not successful, and so the following procedure was used. Conidia were suspended in a nutrient solution containing V8 juice (100ml l^{-1}), sucrose (50g l^{-1}), glycine (1.0g l^{-1}), potassium dihydrogen phosphate (1.0g l^{-1}), magnesium sulphate (0.5g l^{-1}) and Tween 80 (0.1 ml l^{-1}), and the suspensions were adjusted to 10^4 conidia ml^{-1}. Sterile discs (6mm diam) of Whatman 1 filter paper were impregnated with the suspensions and placed on leaves of 4-week-old tomato plants (cv.Eurocross A) that had been sprayed to run off with water or with iprodione of various concentrations. The tomato plants were kept in a humid atmosphere in plastic sheeting cloches in an unheated glasshouse, and the sizes of lesions that developed were measured after 4 days .

The conidial progeny of lesions were assessed in a separate experiment. Tomato plants were inoculated and placed in cloches as above, and the impregnated paper discs were removed after 2 days. Conidia that developed on the lesions after 5 days were suspended in Tween 80 solution containing 50µg streptomycin ml^{-1} and analysed as described below to determine the proportions of dicarboximide-resistant : dicarboximide-sensitive progeny.

The conidia used to impregnate the paper discs were taken from homokaryotic sensitive (HOM-S), homokaryotic resistant (HOM-R) and heterokaryotic (HET-S2 and HET-R2) colonies. The HET-S2 colonies, grown on dishes of MM, gave 5-10% dicarboximide-resistant progeny. The HET-R2 colonies, grown on MM amended with 10µg iprodione ml^{-1}, gave 75-80% dicarboximide-resistant progeny.

Analysis of conidial progeny of heterokaryons

Conidia were suspended in Tween 80 solution, the suspensions were adjusted to 2 x 10^3 conidia ml^{-1}, 0.1ml samples were spread on dishes of MS, and the dishes were incubated in darkness at 25°C for 18h. Germinated conidia were transferred to dishes of MM amended with 25µg vinclozolin or iprodione ml^{-1} and incubated for 4 days at 25°C to determine whether they were resistant or sensitive to the fungicide. The relative proportions of dicarboximide-resistant : dicarboximide-sensitive progeny is a reflection of the relative proportions of mutant : wild type nuclei in the mycelia of heterokaryotic isolates. However the proportions of dicarboximide-resistant : dicarboximide-sensitive monoconidial progeny are not identical to proportions of mutant : wild type nuclei in the mycelia because B.cinerea conidia are multinucleate.

RESULTS AND DISCUSSION

TABLE 1

Differences in growth rate on synthetic media (increase in colony diam mm $24h^{-1}$, mean of 3 replicates) and resistance to dicarboximide fungicides (ED_{50} µg fungicide ml^{-1} MM) between isolates of B.cinerea.

Isolate	Medium			Fungicide	
	MM	CM	MM+0.5M NaCl	Iprodione	Vinclozolin
Wild type HOM-S	11	17	11	0.2	0.8
Mutant HOM-R	4	3	0	>50	>50

The homokaryotic mutant isolate, HOM-R, was very resistant to dicarboximide fungicides but compared to the homokaryotic wild type isolate, HOM-S, grew poorly on synthetic media, especially on media of high osmolarity such as MM containing sodium chloride (Table 1). Mutant HOM-R colonies produced approximately 99% fewer conidia than wild type HOM-S colonies (3×10^4 and 5×10^6 respectively). The phenotypes of heterokaryotic colonies varied, depending on their growth medium and, consequently, on the proportions of mutant to wild type nuclei in the mycelia (see later).

TABLE 2

Changes in % dicarboximide-resistant conidial progeny during growth of a heterokaryotic isolate of B.cinerea on MM.

Isolate	Distance grown, mm from inoculum						
	0	10	40	110	330	550	770
HET-R1	(98)	71	25	-	-	-	-
	(98)	62	48	-	-	-	-
	(98)	-	-	34	3	<1	0
	(98)	-	-	6	1	<1	0

TABLE 3

Changes in % dicarboximide-resistant conidial progeny during growth of a heterokaryotic isolate of B.cinerea on MM amended with 2.5µg vinclozolin ml^{-1}.

Isolate		Distance grown, mm, from inoculum					
	0	10	20	50	70	110	330
HET-S1	(0.5)	99	97	99	99	-	-
	(0.5)	96	96	97	99		
	(0.5)	-	-	-	-	97	98

There was a rapid selection in favour of hyphae containing wild type nuclei when the heterokaryotic isolate HET-R1 was grown on MM (Table 2), and extremely rapid selection in favour of hyphae containing mutant nuclei when the heterokaryotic isolate HET-S1 was grown on MM amended with vinclozolin (Table 3). For example, after 40mm (about 4 days) growth of HET-R1 on MM the proportions of resistant progeny had fallen from approximately 98% to 25-48% and the growth rate had increased from 4mm to 11mm $24h^{-1}$ (a rate similar to that of the sensitive homokaryon, HOM-S). HET-R1 isolates grown on MM eventually became homokaryotic, due to the loss of mutant (dicarboximide-resistant) nuclei, after 770mm (about 100 days) growth. After 10mm (about 2 days) growth of HET-S1 isolates on MM amended with vinclozolin, the proportions of resistant progeny had increased from approximately 0.5% to 96-99% and the growth rate had decreased from 11mm to 4mm $24h^{-1}$ (a rate similar to that of the resistant homokaryon, HOM-2). Due to technical difficulties, it was not possible to determine whether prolonged growth of HET-S1 on MM containing vinclozolin would eventually cause loss of wild type nuclei from the mycelia.

Results of the in vivo experiments are given in Tables 4 and 5.

TABLE 4

Size (mm diam after 4 days, mean of 60 replicates) of lesions on leaves of iprodione-treated tomato plants inoculated with conidia from homokaryotic and heterokaryotic isolates of B.cinerea.

Inoculum	Iprodione treatment (proportion of field rate)				
	0	1/8	1/4	1/2	1
HOM-S (0% resistant progeny)	17	2	0.5	0	0
HOM-R (100% resistant progeny)	6	6	5	6	6
HET-S2 (5-10% resistant progeny)	17	3	2	1	2
HET-R2 (75-80% resistant progeny)	8	6	7	6	6

TABLE 5

Proportions of dicarboximide-resistant conidial progeny (mean of 9 replicates) from lesions on leaves of iprodione-treated tomato plants inoculated with conidia from heterokaryotic isolates of B.cinerea

Inoculum	Iprodione treatment (Proportion of field rate)	Resistant progeny (% of total)
HET-S2 (5-10% resistant progeny)	0 (water)	1
	½	95
	1	No conidia
HET-R2 (75-80% resistant progeny)	0 (water)	47
	½	94
	1	No conidia

Conidia from the homokaryotic wild type isolate HOM-S produced large lesions on the untreated leaves, small lesions on 1/8 and 1/4 rate treated leaves and no lesions on leaves that had been sprayed with 1/2 and full field rate iprodione (Table 4). In contrast, conidia from the homokaryotic mutant isolate HOM-R produced significant lesions on all leaves, including those sprayed with iprodione at the full field rate of 1.0g l^{-1}. Results with conidia from the heterokaryon HET-R2 and the homokaryotic mutant HOM-R were not significantly different (Table 4). Results with conidia from the heterokaryon HET-S2 and the homokaryotic wild type HOM-S were similar, except that the HET-S2 conidia produced small lesions on leaves sprayed with iprodione at 1/2 and full field rate.

There were no conidia after 5 days on the lesions produced by HET-S2 and HET-R2 inocula on leaves sprayed with iprodione at full field rate (Table 5). The lesions on untreated leaves, and on leaves sprayed with iprodione at 1/2 field rate, gave rise to both dicarboximide-resistant and dicarboximide-sensitive progeny (Table 5). This result implies that both mutant (dicarboximide-resistant) and wild type (dicarboximide-sensitive) nuclei were present in the mycelia within the lesions on untreated and iprodione-treated leaves. The proportions of dicarboximide-resistant progeny produced by the lesions differed substantially from those produced by the inocula from which the lesions had developed. On untreated leaves, the lesions produced fewer resistant progeny than the parent inocula, and on iprodione-treated leaves the lesions produced more resistant progeny than the inocula (Table 5). This result indicates that there had been selection in favour of hyphae containing mutant nuclei on iprodione-treated leaves. However, selection did not cause the complete loss of either kind of nucleus from the lesions.

This report gives results of a study of one heterokaryon, studies of other resistant heterokaryotic isolates have given very similar results. The *in vitro* and *in vivo* experiments exemplify the potential of heterokaryons for maintaining dicarboximide-resistant nuclei, in populations of *B.cinerea*, in the presence and absence of selection pressure from

dicarboximide sprays. However, surveys of B.cinerea populations in commercial glasshouses (Summers, unpublished) showed that resistant heterokaryotic isolates are rare, and that when resistant isolates are present, they are predominantly homokaryotic mutants with low levels of resistance to the dicarboximides (ED_{50} < 5µg fungicide ml^{-1}). This suggests that heterokaryosis may not be an important mechanism for maintaining dicarboximide-resistance under field conditions.

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SENSITIVITY OF BARLEY POWDERY MILDEW TO SYSTEMIC FUNGICIDES IN THE U.K

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ABSTRACT

UK powdery mildew populations on spring barley were tested for sensitivity to the three systemic fungicides, ethirimol, triadimenol and fenpropimorph. The data collected in the 1984 survey indicated that sensitivity to ethirimol has increased in recent years, whilst triadimenol sensitivity has decreased. Changes in sensitivity could not be related to differences in disease control. No significant differences in fenpropimorph sensitivity between populations were determined.

INTRODUCTION

The systemic fungicides currently available to control powdery mildew of cereals are divided into three distinct categories as determined by their different cross-sensitivity relationships. These relationships have been examined by Hollomon (1982) and on the basis of his findings we may delineate the hydroxypyrimidines, the C14-demethylation inhibitors (triazoles, imidazoles, pyrimidines and piperazines) and the morpholines.

In order to determine the extent of variation of sensitivity to these different classes of fungicides a survey of barley powdery mildew populations in the U.K was undertaken. Mildew populations collected throughout the U.K. over the period May to July 1984 were assayed in the laboratory for sensitivity to ethirimol, triadimenol, and fenpropimorph. Levels of disease were assessed at the collection sites. For the purposes of this survey mildew populations were taken only from spring barley cultivars in order to allow us to examine relationships between seed treatment and sensitivity changes, initially in the absence of the influence of foliar sprays. The accuracy of test methodology and the influence that subculturing has on sensitivity determination were also examined. In this way the accuracy of survey data were validated.

This type of survey provides a measurement in time of a dynamic situation. Its predictive value is limited. However it does provide basic information to which future distributions of sensitivity can be related. This may assist in the determination of judicious fungicide strategies.

MATERIALS AND METHODS

Collection of field populations

Populations of powdery mildews were taken from a representative distribution of spring barley fields in England. Fewer fields were sampled in Scotland. East Anglia, traditionally an area of high cereal production

was sampled most extensively. The cultivar Triumph dominated the survey (73% of fields), with populations being taken from twelve different varieties in total. Forty-one percent of fields received a systemic seed treatment (37% triadimenol, 4% ethirimol).

In order to gather a representative 'average' population from each field whole leaf pieces infected with powdery mildew were sampled every 15 to 30 metres along one or two field transects. A perimeter area of 30 metres around each field was discarded for sampling purposes. Disease assessments were made at 25 points along the transects. The percentage area of the lowest green leaf covered by active disease was recorded. At earlier growth stages during tillering, leaves were taken from the middle of the crop.

Isolates were sealed in air-tight polythene bags and placed in a portable incubator at 10 to 15°C prior to transfer to optimal growth-room conditions. Alternatively they were despatched in padded envelopes by post to Jealott's Hill. Few problems were experienced with the collection and maintenance of isolates in this manner.

Where fields were sampled twice, first samples were taken between GS14 and GS39 prior to mildew spray applications. Second samples were taken between GS51 and GS71 when most fields had received a spray treatment. Only results from the first sample are reported in detail here.

Laboratory culture of field populations

Populations were cultured on untreated barley leaves, placed on tap water agar in petri dishes. Usually sufficient mildew was collected to establish three dishes per field. Populations were maintained at 19°C under 'daylight' fluorescent light (approximately 7000 lux, 16 hours per day) subcultured every 7 to 10 days and tested when sufficient spores were available. For survey populations this was usually after one generation and never more than three generations.

Sensitivity determination

Ethirimol sensitivity

The method described by Shephard et al (1975) was employed with a number of minor technical modifications. Populations were graded between 0 (least sensitive) and 20 (most sensitive) based on their ability to infect pieces of 8 day old barley prophyll cv Proctor, grown from seed treated with a range of ethirimol concentrations (0, 100, 250, 500, 2000 and 8000ug ai/g seed) applied as 'Milstem' (58% ethirimol ww). Pieces of prophyll were mounted in square petri dishes (5 replicates per concentration) prior to inoculation with 24-48 hour old spores using a small settling tower. Disease assessments were made after 8 days incubation at 19°C.

Triadimenol sensitivity

Environmental conditions and test procedures were as above. Seed was dressed with triadimenol ('Baytan F', 25% triadimenol, 3% fuberidazole, ww) at rates of 0, 2, 10, 30, 60 and 100ug ai/g seed. Sensitivity grades again range from 0 to 20.

Fenpropimorph sensitivity

Fenpropimorph was applied to 7 day old barley prophylls as Corbel (75% fenpropimorph wv). Seedlings were sprayed to maximum retention using a deVilbiss spray gun at rates of 1 and 5ppm ai. They were returned to a controlled environment room prior to being mounted in test dishes after a further day. Sensitivity grades thus range from 0 to 8.

All plant test material was grown in the following environment : day 20°C RH 70%; night 15°C RH 95%; daylength 16 hour, 7500 lux. Test procedures, particularly disease grading were constantly monitored for concordance between personnel in order to minimise 'observer test error'.

Determination of test accuracy

Standard isolates were included in all test batches. These isolates had previously been tested over a period of nine months and had exhibited only random variation in test score. The performance of the standards over a 30 batch period provided a good estimate of error between tests. Error within test was estimated by replicating these and other standards (usually single pustule isolates) 5 to 10 times in the same batch.

Populations, sensitivity tested at generation 0 (direct from field leaf pieces), were re-tested after subsequent subcultures. Also a number of populations collected in 1983 were periodically tested, test results being available to generation 46. In this way short-term and long-term subculture effects on sensitivity were analysed.

RESULTS

The error between tests for fenpropimorph sensitivity exceeded that of the survey sample after arc-sin transformation of the data, indicating that there were no significant differences in fenpropimorph sensitivity amongst the populations tested. Further testing of the least sensitive populations, using the biochemical methods outlined by Hollomon (1982) confirmed this observation. Our inability to measure any significant variation in U.K. populations to fenpropimorph does not preclude the existence of spores in the population with reduced sensitivity to this and other morpholine compounds, though at present their frequencies must lie below that of our limit of detection.

Test errors did not account for the extent of variation measured in the survey sample with regard to ethirimol and triadimenol sensitivity.

Analysis of subculture effects revealed no statistically significant changes in either ethirimol or triadimenol sensitivity from generation 0 to generation 1 (see Table 1). However a statistically significant decrease was noted over the period generation 0 to generation 5. This interesting observation may reflect an increase in the relative fitness of less sensitive spores under optimal laboratory conditions. Regression analysis of 79 populations collected in 1983 and subcultured up to a maximum of 46 generations (25 populations subcultured >20 times) revealed a statistically significant increase in ethirimol sensitivity with time, at a rate of 0.06 units/ generation. A non-significant increase in triadimenol sensitivity was noted at 0.02 units/generation. Initial mean sensitivity

of long-term subculture isolates was 10.4 for triadimenol and 13.4 for ethirimol. Taken together these data suggest that survey sensitivity data are representative of the field situation.

TABLE 1

Sensitivity changes with subculture

	Change in mean sensitivity [1]	
	Triadimenol	Ethirimol
Generation 0 to 1	+ 0.45 (26)	+ 0.67 (17)
Generation 0 to 5	- 1.2 (51)	- 2.1 (43)

[1] Units are based on the 0-20 scale described previously. Figures in brackets represent the number of populations analysed.

The values for ethirimol sensitivity in the present survey in England (96% of field sampled have not received an ethirimol seed treatment) (Table 2,K) are higher than those recorded in previous surveys. Over the period 1974-75 sensitivities recorded from untreated fields in East Anglia had a mean of 13.0, whilst in the summer of 1973 sensitivities were as low as 11.9 (Bent 1978).

In Scotland such an increase in sensitivity with time is not apparent; 1974-75 mean 13.5, compared to 1984 value 12.6. This noteable difference between England and Scotland may reflect relative differences in selection pressure. Lower sensitivity values were recorded in treated fields as observed previously by Shephard et al (1975).

In 1984 disease control by ethirimol at population sensitivities around 12 was good. In three trials on the West coast of Scotland ethirimol maintained 81% disease control up to GS37, against a background of population sensitivities ranging from 11.5 to 12.9.

Triadimenol sensitivity values for the 1984 survey were significantly lower than a group of eight single pustules isolates collected prior to 1980 ($P<0.0001$). The sensitivity of this latter group of isolates ranged from 14.3 to 18.0 with a mean value of 16.3 (compare with values in Table 2). In contrast to ethirimol sensitivities, no significant differences were apparent between treated and untreated fields. Few significant differences were apparent between areas (Table 2) though sensitivity values recorded in Scotland (area A) were significantly higher than England (area K) ($P<0.01$). In England sensitivities determined from populations collected at later growth stages (GS59-GS71), were not significantly different to earlier samples; mean = 7.3, 52 populations. Changes in triadimenol sensitivity (relative to pre 1980 isolates) were not reflected by a breakdown of disease control. A mean of 1.6% disease, (triadimenol treated fields), compared to 6.0% (untreated fields) at periods of time up to growth stage 37 confirm this. No significant correlations were established between ethirimol sensitivity and triadimenol sensitivity, nor was it possible to establish correlations between sensitivity values and disease control.

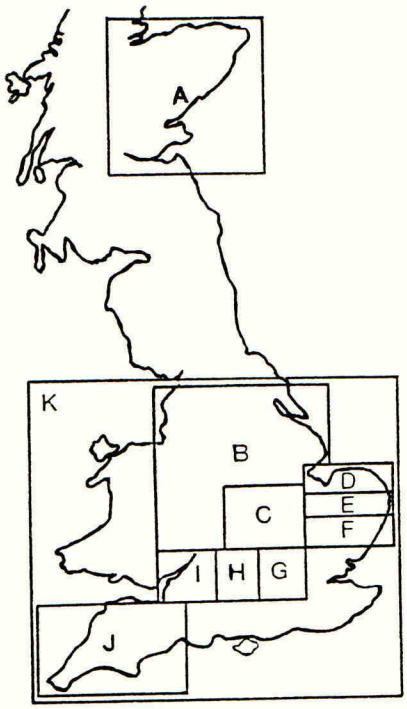

Fig 1. Area designations, for survey sampling points.

TABLE 2

Fungicide sensitivity distributions by area

Area	Triadimenol Mean ± SD	Ethirimol Mean ± SD	Fenpropimorph Mean ± SD
A	7.8±1.9 (12) a	12.6±2.9 (12) a	6.4±1.6 (12) a
B	6.2±2.5 (21) ab	15.2±2.1 (6) ab	7.0±1.8 (4) a
C	6.5±2.7 (13) ab	15.2±3.5 (13) ab	6.8±1.7 (11) a
D	6.3±2.5 (32) ab	15.8±2.2 (32) b	6.9±1.0 (28) a
E	6.4±2.7 (28) ab	15.7±2.9 (28) b	7.1±1.4 (24) a
F	5.1±3.0 (17) b	13.9±3.5 (17) ab	6.9±1.5 (14) a
G	6.1±2.6 (21) ab	14.8±2.6 (21) ab	6.3±1.6 (15) a
H	7.1±2.8 (20) ab	14.1±3.8 (20) ab	7.1±1.3 (16) a
I	7.0±2.8 (8) ab	16.4±2.1 (8) b	6.2±1.7 (8) a
J	7.8±3.5 (17) a	14.9±2.9 (17) ab	6.8±1.8 (13) a
K	6.5±2.8 (162)	15.1±3.0 (162)	6.8±1.4 (133)

Vertical columns with letters in common are not significantly different ($P<0.01$). Figures in brackets are numbers of fields sampled in each area.

DISCUSSION

The increased levels of ethirimol sensitivity now apparent in English populations are not unexpected in view of earlier findings by Shephard et al (1975) that changes in ethirimol sensitivity can respond rapidly in either direction to changes in selection pressure. Whilst sensitivity has increased, sufficient heterogeneity appears to have been maintained to produce measurable selective responses over a short-time period. In Scotland sensitivity values appear a little lower, though disease control has been maintained. Contributory factors towards this position may have been the use of molecules in alternative cross-sensitivity groups, as seed treatments and foliar sprays.

Sensitivity to triadimenol (and by inference other C14-demethylation inhibitors) has changed significantly since their introduction in 1978. It is not possible from the data presented here to determine whether these changes have affected disease control. Current levels of mildew control provided by triadimenol seed treatment appear acceptable. Changes in triadimenol sensitivity in response to selection pressure are not as marked as those exhibited by ethirimol. Prediction of future changes in frequency of the less sensitive spores in the population (able to develop on seedlings grown from 100ppm treatments) is difficult. No trends were apparent between the earlier and later sampling periods.

Optimistic signs that populations may respond to reduced selection pressure can be seen in Scotland, where morpholine sprays have increased in frequency. Although no sigificant variation in fenpropimorph sensitivity is detectable at present, further monitoring would seem prudent in view of the probable increase of morpholine use. Selection pressure on individual fungicides may be reduced by the use of mixtures of one or more of the fungicides. Commercial triazole/morpholine mixtures are already available and the triazole/ hydroxypyrimidine seed treatment (FF4050) described by Northwood et al (1984) provides another means of reducing selection pressure on the demethylation inhibitors. This latter treatment has shown superiority to triadimenol in terms of disease control and yield increase in a recent field evaluation programme. This extra diversity should benefit individual fungicides, though judicious use of the chemicals available will require monitoring of fungicide sensitivity, as populations respond to such innovations.

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DYNAMICS OF TRIAZOLE SENSITIVITY IN BARLEY MILDEW, NATIONALLY AND LOCALLY

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ABSTRACT

From 1981 to 1984, the population of Erysiphe graminis f. sp. hordei on barley in England became increasingly less sensitive to triazole fungicides. Surveys of individual fields of winter barley untreated or treated with triadimenol revealed differences in the frequencies of genotypes of the pathogen sensitive and insensitive to the fungicide which were most marked early in the autumn. By the following spring, the pathogen populations in the treated fields had become more sensitive, probably due to selection, and those in the untreated fields had become less so, probably due to immigration, so that the differences between the fields were less obvious. In the treated fields the sensitive and insensitive fractions of the pathogen population had distinct spectra of pathogenicity characters. The results underline the need for prudence in the use of particular fungicides.

INTRODUCTION

Previous surveys in the UK (Fletcher & Wolfe, 1981; Wolfe et al., 1982,3,4) revealed a gradual decrease in sensitivity to triazole fungicides in the barley mildew population (Erysiphe graminis f. sp. hordei). Much of the data was obtained by monitoring the air spora with a wind impaction spore trap (WIST: Wolfe et al., 1981; see also, Limpert & Schwarzbach, 1981), which indicates general trends but not the underlying complexities. During 1983/4, therefore, a survey was made of changes in the pathogen populations in five winter barley fields, three of which had been sown with triadimenol-treated seed.

MATERIALS AND METHODS

1) WIST surveys

The method was unchanged from the previous surveys. Seedlings of the susceptible barley variety, Golden Promise, were grown in the glasshouse from seed, untreated or treated with 0.025, 0.075 or 0.125 g a.i. triadimenol per kg seed. They were exposed at the first leaf stage for 50 km sections of a standard 200 km route to the east and south of Cambridge. After 7 to 8 days incubation, the numbers of colonies on each leaf were counted and standardised against a value of 100 for the untreated seedlings, to allow comparisons between exposures.

2) Field surveys

Five separated fields of winter barley (two of Maris Otter and one of Tipper treated with triadimenol; two of Tipper not treated) on a farm near Cambridge were sampled on several occasions between October 1983 and July 1984. Random samples of spores were obtained on untreated Golden Promise seedlings, in pots held in a rigid wire cage, which was dragged between rows of the crops. This action disturbs spores within the canopy, some of which are deposited on the seedlings. Following incubation of the trap seedlings, random sets of single colonies were isolated for subsequent testing.

Tests of the single colony isolates were made on detached leaf segments of seedlings grown from seed treated at 0.025, 0.075, 0.125, 0.250 and 0.375 g a.i. triadimenol per kg seed, the last being the recommended commercial rate. Comparisons of samples were made using ED50 values calculated separately for each isolate. Some isolates were also tested on detached segments of seedling leaves of the differential varieties Julia (M1g), Midas (M1a6), Hassan (M1a12), Lofa (M1v) and Ark Royal (M1k + M1a7), representing the current most commonly used resistance genes in spring barley varieties. Comparisons were made between colony numbers relative to those formed on the control, Golden Promise.

RESULTS

1) WIST surveys

The general trend towards triazole-insensitivity in the pathogen population continued (Fig. 1). The data for 1984 at the time of writing included only the first six months.

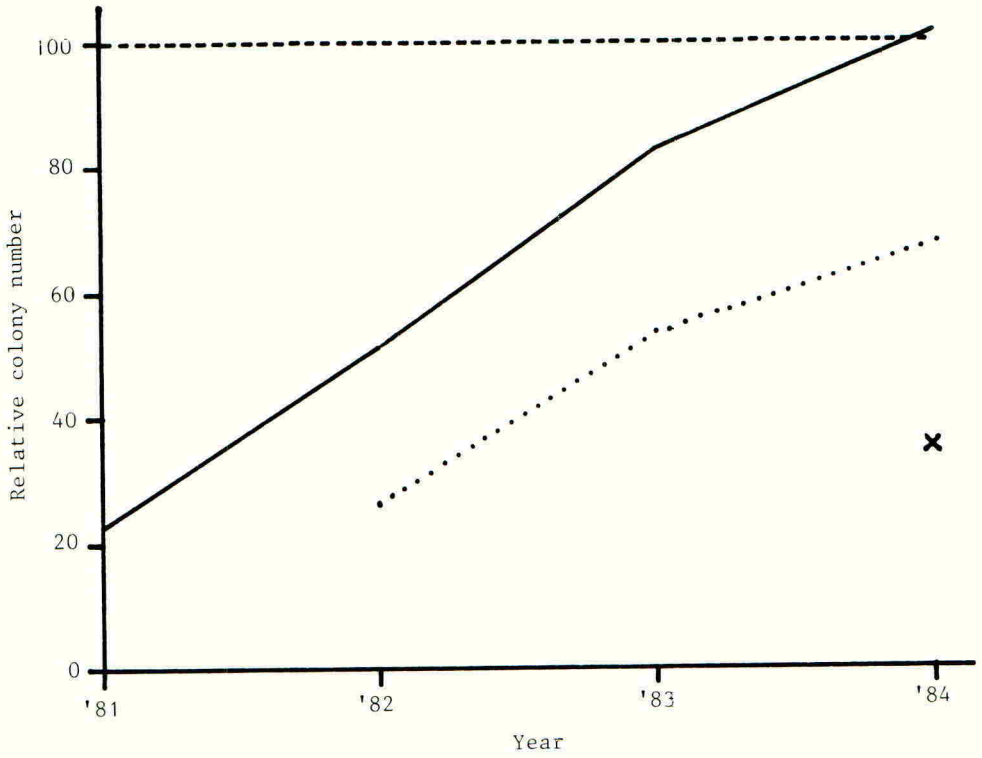

Figure 1. Relative numbers of colonies on seedlings of Golden Promise untreated (---) or grown from seed treated at 0.025 (——), 0.075 (•••), or 0.125 (x) g a.i. triadimenol per kg seed exposed in the WIST in each of four years.

The trends in Fig. 1 indicate that the pathogen is now largely insensitive to a treatment of 0.025 g a.i. per kg seed. Surveys made on other routes indicated that this pattern was widespread, except possibly in the south-west of the country which may have been influenced by the early summer drought of 1984.

2) Field surveys

The ED50 values of the single colony isolates were classified into three discrete categories, sensitive (0-0.01), intermediate (0.01-0.11) and insensitive (> 0.11). There was considerable variation within the intermediate group up to ED50 values of 0.07, but there were few isolates at at the higher end of the distribution. There was less variation among the 'insensitive' isolates which mostly had ED50 values in the range 0.17-0.22.

The distributions of isolates between fields and dates, classified by ED50 value, is given in Table 1. For the earlier dates, the mean values are based on tests of 40 to 90 isolates per field at each date; the observations on the last date were based on seven isolates per field.

TABLE 1

Mean ED50 values for single colony isolates of E. graminis f. sp. hordei obtained from five fields of winter barley on different occasions during 1983-4.

a) untreated fields

Date	Tipper (1) sens.	int.	ins.	Tipper (2) sens.	int.	ins.
7/11	100	0	0	66	30	0
29/11	42	59	0	42	56	3
29/3	57	44	0	29	70	0

b) treated fields†

Date	M. Otter (3) sens.	int.	ins.	Tipper (4) sens.	int.	ins.	M. Otter (5) sens.	int.	ins.
26/10	-	-	-	40	40	20	-	-	-
7/11	60	40	0	53	38	11	24	50	26
29/11	44	50	6	44	45	11	58	41	2
4/1	-	-	-	41	49	10	24	73	3
29/3	0	100	0	14	86	0	0	100	0

† Fields grown from seed treated with triadimenol (0.375 g per kg seed)

In early autumn, there was a greater proportion of more insensitive isolates in the treated than in the untreated fields (Table 1). As time passed, the differences between the treated and untreated fields diminished, partly because of a decrease in the 'insensitive' isolates in the treated fields, and partly because of a trend towards decreased sensitivity in the untreated fields.

There were also changes in pathogenicity of the populations during the spring (Table 2) in that four of the five pathogenicity characters increased in frequency in all fields.

TABLE 2

Relative frequencies of various pathogenicity characters in E. graminis f. sp. hordei in five winter barley fields during spring 1984.

Date	Pathogenicity versus: Julia Mlg	Midas Mla6	Hassan Mla12	Lofa Mlv	Ark Royal Mlk/a7
29/3	59	27	9	39	45
5/6	92	59	28	37	65

Pathogenicity tests were also made on representative isolates from the fungicide 'sensitive' and 'insensitive' classes from treated fields 4 and 5 taken on 4/1/84 (Table 3).

TABLE 3

Mean pathogenicity of 'sensitive' and 'insensitive' isolates from fields 4 and 5, sampled on 4/1/84.

Field no. and var.	Julia Mlg	Midas Mla6	Hassan Mla12	Lofa Mlv	Ark Royal Mlk/a7	ED50
a) 'sensitive' isolates						
4.Tipper	15	18	3	7	42	0.01
5.M. Otter	18	24	0	7	35	0.01
b) 'insensitive' isolates						
4.Tipper	25	28	6	51	0	0.19
5.M. Otter	22	38	21	59	0	0.24

In Table 3, pathogenicity for all differentials except Ark Royal was higher among the 'insensitive' than among the 'sensitive' isolates, particularly so for pathogenicity on Lofa. Pathogenicity for Ark Royal, on the other hand, was high among the 'sensitive' isolates but absent among the 'insensitive' isolates.

DISCUSSION

The continuing increase in level and distribution of greater insensitivity to triazole fungicides in the pathogen population reflects the continuing use of these compounds on a very large scale in England. Some of the complexity underlying these changes became apparent from the field surveys, for example, in the range of variation particularly among isolates showing intermediate levels of insensitivity. Such variation could result from the interaction of a number of genes affecting pathogen response to the

fungicides. This would be consistent with the data of van Tuyl (1977) who recognised at least eight loci involved in moderate degrees of insensitivity to imazalil in Aspergillus nidulans. Indeed, following the argument of Georgopoulos (1984), it is conceivable that some of the same mechanisms and genes may be operating in A. nidulans against imazalil and in E. graminis against triadimenol.

In the field surveys, isolates with high insensitivity decreased in frequency during the winter and spring. Their high initial frequency was presumably due to selection in the early autumn when the leaf concentration of triadimenol was at a maximum. Subsequently, there was probably a much longer period during which isolates with intermediate insensitivity could be selected without any requirement for a high level of insensitivity. Clearly, however, there must be a considerable risk attached to repeated applications of the same fungicide to a particular crop, which would extend the period of selection for a high level of insensitivity relative to that for intermediate levels.

There was a general trend towards a reduction in the frequency of sensitive isolates over all fields. In the untreated fields this must have been due, presumably, to immigration of spores carrying moderate insensitivity into those fields from late autumn onwards. With the exception of field 4, there was little infection in any of the fields after the autumn so that the influence of immigrant infections would be relatively large and easily detectable. The data from the general survey (Figure 1) indicate that immigrant spores would be likely to carry intermediate insensitivity.

Further support for the hypothesis of immigration comes from the marked changes in pathogenicity in all fields (Table 2). Neither Maris Otter nor Tipper possess resistance genes that select for the major pathogenicity characters in current spring barleys so that, in the absence of migration, it would be expected that the frequencies of recognised pathogenicity characters would decline rather than increase with time. It was also noted that the highest frequency of pathogenicity for Ark Royal in the spring occurred in the winter barley field that was closest to a field of Triumph, a susceptible spring variety, that produces spores pathogenic for Ark Royal.

The net result of selection and migration in the winter barley fields was that all five pathogen populations tended towards intermediate sensitivity in the spring. Superficially, this is analogous with the end result of selection for insensitivity to ethirimol during the mid-1970's, described by Brent et al. (1982), and it may be tempting to consider that the pathogen is now moving to some acceptable equilibrium position of intermediate insensitivity to the triazoles. However, the current use of triazole fungicides is much greater than was that of ethirimol, and winter barley is now much more common than during the early 1970's. In our view, therefore, the situation is not stable and continued emphasis on triazole programmes might lead to further deterioration in fungicide performance.

There was a linkage disequilibrium involving the five pathogenicity characters and triazole insensitivity (Table 3). The similarity between the populations in the two fields may indicate separate common origins of the sensitive and insensitive fractions, perhaps even as single clones. The higher values of pathogenicity for Midas associated with the insensitive population fractions were consistent with previous observations of a linkage disequilibrium involving these two characters (Wolfe et al., 1983, 1984).

The major implications of the observations are that growers should minimise their use of particular fungicides and that they should diversify between materials having different modes-of-action (anon. 1984), both in space and in time. Ideally, varieties highly susceptible to mildew should not be grown and different sets of resistance genes should be incorporated in winter and spring barley varieties. The observations also confirm the logic of integrating fungicide use into variety mixtures by treating only one component with a fungicide (Wolfe, 1981; Wolfe and Riggs, 1983). This technique enhances disease control and maximises yield while minimising cost. Most importantly, the period of selection for a high level of fungicide insensitivity, and the leaf area on which this occurs, are also minimised.

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EFFECTS OF FUNGICIDE REGIMES ON SENSITIVITY AND CONTROL OF BARLEY MILDEW

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ABSTRACT

Seed treatments and sprays of pyrimidine and triazole fungicides were applied to barley cv. Golden Promise. To measure fungicide sensitivity in field populations of mildew, barley seedlings grown under controlled conditions from fungicide-treated seed were placed in the field plots to collect inoculum, transferred to a glasshouse, and subsequently assessed for mildew.

In 1982 and 1983 all treatments at recommended rates decreased field mildew greatly and increased yield. Despite variability in test results, some small single-season shifts in sensitivity were detected in both years. An ethirimol seed treatment or spray decreased ethirimol sensitivity. To a lesser extent repeated triazole treatment tended to reduce triadimenol sensitivity especially at full rather than reduced rates. An ethirimol-triadimefon tank-mix, and generally also mixed programmes where a seed treatment and subsequent spray contained different fungicides, caused no detectable change. There were no consistent differences between a seed treatment and a spray application of the same fungicide, in their effects on sensitivity. Indications of negative cross-resistance between ethirimol and triadimenol were obtained, each fungicide tending to induce increased sensitivity towards the other.

INTRODUCTION

The factors that determine the occurrence and rate of increase of fungicide resistance in crop pathogens are not well understood. From theoretical indications and from practical experience, it is generally agreed that the more frequent application of fungicides increases the risk of resistance. It is commonly considered that mixed application programmes or use of mixed formulations, involving two or more fungicides with different mechanisms of action, will delay resistance. However, there is very little evidence from field experiments for such effects. There are also important questions such as the relative risks posed by seed treatments and sprays, and the effects of different rates of application, about which no clear consensus of opinion exists.

Observations of decreased sensitivity of barley mildew populations have been reported after use of ethirimol (e.g. Wolfe 1975, Shephard *et al.* 1975) and triazole fungicides (e.g. Fletcher & Wolfe 1981, Hollomon & Butters 1981). We applied fungicides of the triazole and pyrimidine groups, which control powdery mildew by different mechanisms of action, to plots of spring barley, as seed treatments and sprays in different combinations and at different rates of application. We then attempted to measure any subsequent changes in sensitivity of mildew, by using a bait plant technique which collects fresh inoculum direct from field plants.

MATERIALS AND METHODS

Fungicide treatments in the field

Fungicides were applied in 1982 and 1983 to 12m x 12m plots of cv.

Golden Promise, replicated four times in randomised blocks, at a site near Long Ashton. A 12m wide buffer zone around the experimental area also was sown with Golden Promise, and the rest of the field with Atem, and in these areas mildew was controlled with sprays of pyrazophos (Afugan). Seed was sown at 160 kg/ha on 25 March (1982) and 9 March (1983).

Triadimenol was applied to seed as Baytan (25% powder) at 37.5g a.i./100 kg seed and ethirimol as Milstem (58% w/v s.c.) at 390g a.i./100 kg seed. Sprays were applied at GS 30-31, 37 and 58, with triadimefon as Bayleton (25% w.p.) at full rate (125g a.i./ha) and fractions thereof, triadimenol as Bayfidan (25% s.c.) at 125g a.i./ha, ethirimol as Milgo E (25% s.c.) at 313g a.i./ha, fenpropimorph as Corbel (75% s.c.) at 750g a.i.l/ha, and UK 143 (experimental w.p. containing 25% triadimefon and 25% quinomethionate), at 125g each a.i./ha. Most 1982 fungicide programmes were repeated in 1983, but there were a few changes (Table 1).

Assessment of mildew in the field

Ten plants were taken from each plot and the number of active mildew pustules on three or four uppermost fully expanded leaves counted on main tillers. Total counts on all these leaves were summed, and mean numbers of pustules per tiller calculated.

Fungicide sensitivity

This was assessed on 'bait' plants - barley seedlings (cv. Golden Promise) grown from seed either untreated or treated with triadimenol or ethirimol. In 1982 seeds were treated at 37.5, 25.0, 12.5, 8.0 and 4.0g triadimenol/100 kg seed and at 390g and 97g ethirimol/100 kg seed. Batches of seed were sown weekly, 12 per 9 cm diam. pot, and allowed to grow on a capillary bench in a controlled environment (CE) room at 15°C. After fourteen days the second leaf was expanding and the seedlings were placed in the field; groups of eight pots (7 treatments plus 1 untreated) were positioned at a predetermined site at the centre of each field plot. The test seedlings were left in the field for 1 - 7 days, depending upon the general amount of mildew present in the experimental area, before being transferred to a glasshouse with a filtered air supply. When mildew pustules were fully developed, the number on both surfaces of the second leaf was counted on each of ten seedlings per pot. Fungicide sensitivity was expressed as the ED50 value, i.e. the dose of seed treatment required to decrease infection to half that on untreated seedlings.

In 1983 bait plants were only put out every two or three weeks. They were grown on a dry bench in the CE room and watered overhead. This method was found to improve uptake of the seed treatment. The 4.0g rate of triadimenol was removed and a 62.5g rate added, because incomplete mildew control was given by 37.5g in 1982.

RESULTS

Field data

In 1982 mildew increased to moderate amounts by early June and then greatly declined. All fungicide programmes decreased mildew infection and gave substantial increases in yield figures (Table 1), especially the triadimefon sprays repeated three times, and the single sprays of fenpropimorph or ethirimol-triadimefon tank-mix.

In 1983 disease developed more slowly and peaked towards the end of June. Good control until early June was achieved by all treatments except triadimefon at one-eighth recommended rate. By late June most plots contained substantial amounts of mildew, except those which had received a second spray of triadimefon or triadimenol at full strength. Largest yield increases (up to 37%) were given by the triazole fungicides sprayed three times (even at one-eighth rate), by triadimenol seed treatment alone or followed by a triadimefon spray, by the tank-mix, and by a single fenpropimorph spray.

TABLE 1

Fungicide treatments, mildew infection in field plots, and yields

Treatments[1]		1982			1983		
Seed	Spray[2]	Mildew pustules per tiller		Yield[3] increase	Mildew pustules per tiller		Yield[3] increase
		10/6	30/6	(t/ha)	10/6	27/6	(t/ha)
-	-	131.0	27.3	n.a.	31.4	42.9	n.a.
Tl	-	6.3	8.6	0.61	2.8	26.2	0.99
-	3xTf	0.5	0.1	1.42	4.9	2.8	1.29
-	3xTf($\frac{1}{2}$)	1.2	0.8	0.92	-	-	-
-	3xTf($\frac{1}{4}$)	7.0	0.4	1.19	8.9	22.6	0.75
-	3xTf($\frac{1}{8}$)	-	-	-	16.6	36.8	0.86
-	3xUK143	0.4	0.3	1.37	-	-	-
-	3xTl	-	-	-	1.2	0.3	1.23
-	Tf	1.4	3.2	0.69	5.1	29.5	0.30
Et	-	22.1	5.1	0.57	7.8	26.2	0.38
-	Et	6.0	4.3	0.67	11.7	29.1	0.50
-	Tf/Et	0.5	1.6	1.06	1.1	19.1	1.12
Et	Tf	0.3	0.2	0.82	0.9	14.9	0.79
Tl	Tf	1.5	3.0	0.62	1.5	24.4	1.29
Tl	Et	11.6	8.0	0.52	-	-	-
-	Tl	-	-	-	2.4	30.2	0.65
-	F	0.1	0.2	1.00	1.0	20.2	0.94
LSR[4]:		7.1	4.3	-	5.2	2.9	-
LSD:		-	-	0.60	-	-	0.69

[1] Tl = triadimenol, Tf = triadimefon, Et = ethirimol, F = fenpropimorph, Tf/Et = tank-mix, fractions indicate dose in relation to full rate.

[2] Spray dates: 13/5, 1/6, 14/6 (1982); 19/5, 11/6, 28/6 (1983); single sprays were applied on first date only.

[3] Yields in untreated plots: 4.16 t/ha (1982); 3.46 t/ha (1983).

[4] LSR = least significant ratio for comparing pairs of fungicide treatments (larger value divided by smaller value).

Fungicide sensitivity

In 1982 control of mildew on bait plants was poorer than expected. Only the top rate of seed treatment gave a substantial degree of control, and ED50 values were very erratic. Thus figures for individual times of sampling are not presented. However, when data from all sampling dates were drawn together, there were tentative indications of changes in sensitivity. In several different statistical analyses (an example is given in Table 2) the only change to occur regularly was a clear decrease in ethirimol sensitivity in response to ethirimol seed treatment alone; an ethirimol spray appeared to have a similar but less marked effect. Some analyses also showed significant slight shifts towards triadimenol resistance, and conversely shifts to ethirimol sensitivity (i.e. 'negative cross-resistance'), after repeated triadimefon sprays at the higher rates, or increased triadimenol resistance after a triadimenol seed treatment plus a single triadimefon spray. No shifts were detected in any analysis after application of the fungicide mixtures, the mixed programmes, or the single application of triadimenol seed treatment or of a triadimefon or fenpropimorph spray.

TABLE 2

Sensitivity of mildew populations to triadimenol and ethirimol in 1982

Treatments[1]		Shift in fungicide sensitivity (based on data from 12 successive weekly batches)[2]	
Seed	Spray	Triadimenol	Ethirimol
-	-	0	2.7
T1	-	0.16	- 7.4
-	3xTf	1.85**	-23.1*
-	3xTf($\frac{1}{2}$)	0.98*	-16.8
-	3xTf($\frac{1}{4}$)	0.81	-10.6
-	3xUK143	-0.51	-22.9*
-	Tf	-0.16	-15.7
Et	-	0.08	62.2**
-	Et	0.29	18.0
-	Tf/Et	0.73	5.3
Et	Tf	-0.65	- 2.4
T1	Tf	-0.11	9.6
T1	Et	-0.32	-11.5
-	F	-0.43	-16.4
LSD (5%):		±0.95	±20.4

[1] See Table 1 for abbreviations and spray dates.

[2] Figures are changes per week in ED50 values, calculated from effects at the highest test dose only and expressed as a linear regression over the 12-week sampling period. Mean ED50 values for untreated plots were 24.2g triadimenol and 492.3g ethirimol per 100 kg seed.

* and ** = significantly different (5% and 1% levels) from untreated.

Better dosage-response curves for bait plants were obtained in 1983, although ED50 values were still subject to apparently random variations and high errors. The response of mildew from untreated field plots varied considerably between sampling dates. Results from two individual sampling dates are presented in Table 3; no significant differences between treatments were observed at other times of sampling (17/5, 31/5, 12/7). Despite the large variability, certain trends in fungicide response were apparent. Almost all the indications mentioned above for 1982 were given again, particularly at the second and third times of sampling shown in Table 3. However, a single triadimefon or triadimenol spray gave slight decreases in triadimefon sensitivity, even when applied after ethirimol seed treatment. There were indications of differences between the three rates of repeated triadimefon sprays, with regard both to decreased triadimenol and increased ethirimol sensitivity. The highest rates used appeared to have more marked effects, and the full-strength triadimenol and triadimefon sprays behaved similarly.

TABLE 3

Sensitivity of mildew populations to triadimenol and ethirimol in 1983

Treatments[1]		Fungicide sensitivity (at two sampling dates)[2]			
		Triadimenol		Ethirimol	
Seed	Spray	14/6	28/6	14/6	28/6
-	-	10.7	8.3	47.6	30.0
Tl	-	11.6	8.1	70.3	29.5
-	3xTf	11.7	11.7*	31.8	24.9
-	3xTf($\frac{1}{4}$)	11.0	11.0*	49.5	34.1
-	3xTf($\frac{1}{8}$)	14.2	9.2	46.5	38.7
-	3xTl	9.6	12.8**	29.3	23.5
-	Tf	9.4	13.5**	36.4	37.5
Et	-	10.0	10.4	79.8*	59.8*
-	Et	9.0	11.2*	114.8**	64.4*
-	Tf/Et	12.6	10.0	44.3	31.8
Et	Tf	10.3	11.8*	38.2	40.4
Tl	Tf	15.9*	14.2**	46.3	67.0*
-	Tl	12.1	11.1*	62.2	25.7
-	F	10.2	10.9	33.3	27.3
	LSD (5%):	3.94	2.71	26.99	24.96

[1] See Table 1 for abbreviations and spray dates.

[2] Figures are ED50 values (g/100 kg seed), obtained from plots of log lesion number against triadimenol dose and against $e^{-0.8 \times \text{ethirimol dose}}$. Dates are when test plants were put out to collect inoculum (GS 39 and 58).

* and ** = significantly different (5% and 1% levels) from untreated.

DISCUSSION

The use of bait plants to monitor small changes in fungicide sensitivity presented several difficulties. Adjustments in the watering method

in 1983 helped to give better fungicide uptake (J.A.Pickard and G.A.Carter, unpublished results), and greater and more reliable fungicide responses. Another difficulty was that different fungicide treatments in the field greatly affected the amount of inoculum available to the bait plants, and this probably affected the results of sensitivity assays. Current studies of inoculum-response relations and of appropriate methods of co-variance analysis may reveal ways of correcting for this source of error by more refined statistical analyses.

The changes in fungicide response were small (ED50 values increased 2- or 3-fold), and caused no breakdown of field disease control; they might increase to more serious levels with continued year-by-year use of particular regimes. However, this study was done primarily to indicate relations between strategies of fungicide use and shifts in sensitivity. It has shown shifts in sensitivity induced by repeated application of the same fungicide, the avoidance of shifts by applying mixed spray applications, and the occurrence of negative cross-resistance between triazole and pyrimidine fungicides. The influence of differences in dose of sprays was harder to discern, and warrants further study, but the balance of evidence suggests that high doses tend to induce resistance or negative cross-resistance more readily than lower ones. Differences between seed treatments and sprays sometimes occurred but were irregular in direction. In general, ethirimol sensitivity proved rather more changeable than triazole sensitivity, so that it may be a better 'guinea-pig' in further studies of strategies of fungicide application.

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GENETIC CONTROL OF TRIADIMENOL RESISTANCE IN BARLEY POWDERY MILDEW

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ABSTRACT

Techniques were developed for routine crossing of barley powdery mildew (Erysiphe graminis f.sp. hordei), and the induction of mutants using N-methyl-N'-nitro-N-nitrosoguanidine. The frequency distribution of triadimenol sensitivity of progeny from two crosses was continuous, indicating that resistance is controlled not by one major gene, but probably by a complex genetic system. Similar levels of resistance in different mildew isolates may be derived from different combinations of these factors. Only small changes in sensitivity to triadimenol were achieved by mutagenesis. The results suggest that mildew populations are unlikely to respond rapidly to selection for greater resistance than is present already.

INTRODUCTION

Use of triazole fungicides throughout the U.K. to control barley powdery mildew (Erysiphe graminis f.sp. hordei) has led to some decline in the sensitivity of mildew populations to these fungicides (Fletcher & Wolfe 1981). Several levels of resistance have been identified, which are stable, suggesting that genetic factors are involved. In laboratory tests, LC50 values may differ 1,000 fold between the most sensitive and resistant field isolates (Hollomon 1982), although in greenhouse tests on whole plants differences seldom exceed 25 fold. Isolates resistant to the triazole triadimenol, were cross-resistant to other fungicides that inhibit C-14α demethylation during sterol biosynthesis, but not to morpholine fungicides which act at later steps in this pathway (Butters et al., 1984). Resistant isolates are frequently sensitive to the hydroxypyrimidine ethirimol (Hollomon 1982), and some improvement in the performance of ethirimol ("Milstem") has accompanied use of triazole fungicides in recent years.

This paper extends earlier work on the genetic control of ethirimol resistance (Hollomon 1981), and examines the nature of the genetic control of resistance to triadimenol. We also report techniques for routine crossing of mildew isolates, and induction of mutants.

MATERIALS AND METHODS

Mildew cultures

Cultures were obtained as single pustule isolates from separate field populations. These were maintained on detached primary barley leaves (cv. Proctor, Hollomon 1977), and transferred every 10d to fresh leaf material using a small paint brush. Resistant isolates infected plants grown from seed treated with recommended rates of triadimenol. Bioassay procedures have already been described, and LC50 values are expressed as logarithms ($\log_{10}$ μg ml^{-1}) that have been multiplied by 10^3 to avoid use of bar numbers. Assuming a gene for gene relationship between mildew and its host, the reaction of each isolate used in this work on several differential barley cultivars was used to identify pathogen virulence genes (Table 1). A wider set of differentials was used occasionally as a check on contamination.

Mutants

Mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine (NTG) was based on the method of Gabriel et al. (1982). 10d old Proctor seedlings were inoculated, using a cotton bud, with conidia of isolate 23D5. This isolate was sensitive to both ethirimol and triadimenol, and was known to compete poorly in field populations. After 20h, leaves were cut at their base and placed in 12.5cm test-tubes containing benzimidazole (100ug ml^{-}) and NTG (25ug ml^{-1}) in 2ml water (pH 7.2). The effects of this NTG concentration were somewhat variable, but generally resulted in about 90% kill; lower concentrations were used in some experiments to achieve 50% or more survival. Tubes were left uncapped for 4h under lights to promote transpiration; they were then capped and placed in continuous illumination at 15^{0}C (Gallenkamp IH 30 Incubator) for 4d. Leaves were then cut and laid on 0.5% water agar in clear polystyrene boxes, leaving behind the base which had been immersed in NTG solution. After 6-10d, any mildew on NTG treated leaves was transferred to Proctor leaves grown from seed treated with ethirimol at the recommended rate (4,000 ppm), or triadimenol (47 ppm) at 1/8th of the recommended rate, or leaves of the cultivars Rogers, Maris Concord, H1063 and Wing. The parent isolate, 23D5, produced a few normal pustules on Rogers ($M1a_7$), none on Maris Concord ($M1a_6$), and limited mycelium coupled with extensive browning on H1063 ($M1_k$) and Wing ($M1_k$ + $M1a_7$). In some experiments mildew from NTG treated leaves was increased on untreated Proctor before selection with fungicide, or the differential cultivars. Colonies that developed were maintained on fungicide-free Proctor leaves before re-testing and bioassay. Infected leaves placed in benzimidazole solution only, but otherwise treated identically, were used as controls. Except for the initial inoculation of greenhouse grown plants, all manipulations were performed in the laboratory, and no contaminants were detected. All attempts, over a period of three years, to induce mutants with u.v. light were unsuccessful, and no suitable concentration of the mutagen, ethyl methane sulphonate, was found which did not kill barley leaves before mildew sporulated.

RESULTS

Crosses

In both crosses analysed, segregation of mildew virulence genes showed that recombinants were recovered in the expected frequencies, and that resistance to triadimenol did not affect ascospore viability. The frequency distribution of progeny in each cross was continuous and approximately normal, suggesting that major genes for triadimenol resistance were not segregating. However, genetic control of triadimenol sensitivity was evident, for significant additional variation was released in both crosses. Some progeny from the cross between triadimenol sensitive isolates (DH14 x DH19), were less sensitive than either parent, although differences were small and no progeny resembled the most triadimenol resistant isolates found in field populations (Table 2). Progeny from the cross between resistant and sensitive isolates (JB152 x DH14) were more variable, but the mean LC50 values for both parents and progeny were not significantly different. Although some progeny were more resistant to triadimenol than JB152, differences just failed to reach significance ($p = 0.05$). In this cross progeny virulent on Maris Concord ($M1a_6$) were somewhat less sensitive to triadimenol than were avirulent progeny, but differences were not statistically significant. Unfortunately, few bioassays have been replicated, and estimates of heritability are not yet possible. Attempts to cross compatible triadimenol resistant isolates have so far failed.

TABLE 1

Characteristics of mildew cultures used in these studies

Isolate	Year isolated	Treatment sensitivity LC50 ug ml^{-1}	Treatment sensitivity LC50 log 10 µg ml^{-1} x 10^3	Virulence genes*						
23D5	1973	0.061	1.78	Va	Va_6	va_7	va_{12}	vg	vv	Vk
DH14	1976	0.002	0.30	Va	Va_6	va_7	va_{12}	vg	Vv	vk
DH19	1976	0.008	0.89	va	Va_6	va_7	va_{12}	vg	Vv	Vk
JB152	1982	1.034	3.01	Va	va_6	va_7	va_{12}	vg	Vv	Vk

*Capital V signifies avirulence (see Hollomon, 1981)

Crosses

Crosses between incompatible isolates using techniques described earlier (Hollomon 1981), reliably produced cleistothecia under greenhouse conditions only in spring or early summer. To cross isolates routinely throughout the year suitable growth cabinets were used (Saxton Ltd., Bradbury, Cheshire, U.K.). Six to seven barley seedlings (cv Proctor) were grown for 3 weeks at 20°C, in 13cm diameter pots, in soil-less compost (Eff Products, Guildford, U.K.). During this period compost was moistened solely with water. Light intensity was 30W m^{-2} of visible radiation during a 16h photoperiod. Relative humidity was 70% during both the light and dark period. Only filtered air entered cabinets and contamination by unwanted mildew was not observed. The cabinet was sterilized between crosses with chlorine gas. Inoculum of the two isolates to be crossed was obtained, eight to nine days after inoculation, from plants growing in an isolated propagator (Jenkyn et al., 1973). Equal quantities of conidia of each isolate were collected on glazed paper, mixed with a cotton bud, which was then rolled over the surface of the first and second leaves of the Proctor seedlings. The reaction of each parental isolate on a set of differential barley cultivars was checked at this time.

After inoculation, each pot of seedlings was kept under a polythene bag before its return to the growth cabinet the next day. Seedlings then received Hoaglands solution (100ml) on alternate days, and water as required. After three weeks, when whitish mycelial mats indicated that cleistothecia were forming, the temperature was raised by 0.5°C each day for 12d. Cleistothecia were then removed from senesced leaves and stored for one month at 4°C. To obtain ascospore progeny, cleistothecia were surface sterilized with 1% hypochlorite, rinsed in sterile distilled water, and arranged on moist filter paper. These papers were attached inside the lid of a clear polystyrene box that contained detached leaves lying on water agar (5g litre^{-1}). When kept moist, cleistothecia began to dehisce after 5d at 15°C. The lid was transferred to cover fresh leaves every 5d, and any ascospores that germinated to form colonies were cloned to provide sufficient material for bioassay.

TABLE 2

Triadimenol sensitivity in two crosses between mildew isolates

Cross	No. of progeny analysed	Triadimenol sensitivity Mean LC50 $\log_{10}$ µg ml^{-1} x 10^3 Parental	Progeny F_1	Range of Progeny
DH14 x DH19	30	0.60 ± 0.05	1.55 ± 0.63	0.08 - 2.64
DH14 x JB152	14	1.79 ± 0.84	2.26 ± 1.06	0.01 - 4.10

Mutagenesis

NTG treatment of a triadimenol sensitive isolate (23D5) failed to generate the range of variation in sensitivity to this fungicide encountered in field populations. In fact, isolates have only been recovered after mutagenesis from leaf material grown from seed treated at 1/8th, or less, of the recommended rate of triadimenol ("Baytan"). Just one of these isolates (N91) showed differences by bioassay. The LC50 of this isolate was no more than 10 fold greater than that of its parent, a difference just detectable in our laboratory bioassay. Small decreases in sensitivity to triadimefon, diclobutrazol and propiconazole were also observed, but not to other fungicides that inhibit C-14x demethylation during sterol biosynthesis. On whole plants in greenhouse tests differences between N91 and 23D5 could not be demonstrated. Selection of mutants on leaves from seed treated with ethirimol (4,000 ppm) was also unsuccessful. Treatment with NTG did, however, alter mildew, and some isolates had different virulence characteristics than those of 23D5. Of particular interest were isolates which either lost, or had reduced, virulence, especially on cultivars with Ml_{a_7}, Mlv or Mlg host plant resistance genes.

DISCUSSION

Considerable variation in sensitivity to triadimenol exists in barley mildew populations and, although some of this variation is undoubtedly environmental, much is genetic. The continuous nature of the data from crosses suggests that resistance is not controlled by one gene, but is under more complex control. Differences in the levels of cross-resistance between fungicides that inhibit C-14x demethylation (Hollomon 1982) also indicate that resistance to these fungicides may involve more than one mechanism. Simultaneous changes in a number of genes is unlikely, which may explain the difficulty of altering triadimenol sensitivity by mutagenesis. However, the number of genes involved in triadimenol resistance cannot be determined from these data. The cross between two triadimenol sensitive isolates released significant variation, suggesting that the two parents were not genetically identical, and that triadimenol sensitivity involved a different combination of factors in each isolate.

The genetic basis of triadimenol resistance seems similar to that for ethirimol (Hollomon 1981). In view of the negatively correlated cross-resistance between triadimenol and ethirimol observed in some field isolates, it is interesting that the cross between DH14 and DH19 (both ethirimol resistant isolates) yielded progeny significantly more sensitive

to this hydroxypyrimidine fungicide than either parent. Whether this negatively correlated cross-resistance is a property of the same gene, or simply reflects differences in current use of these two fungicides and selection of independent genes, must await the outcome of both triadimenol and ethirimol assays of progeny from this and other crosses. Data are also required from more progeny to determine if linkage exists between triadimenol resistance and virulence on barley cultivars possessing the Mla_6 host plant resistance gene, as seems likely from field observations (Wolfe et al. 1983).

Some isolates selected for bioassay after treatment with NTG had changes in more than one virulence characteristic, suggesting that mutagenesis lasting several days affected many genes. In these studies we attempted to select isolates for fungicide resistance, yet several isolates showed reduced levels of virulence on certain barley cultivars with specific genes for host plant resistance. Failure in the past to find mutants to avirulence may, indeed, be due to the difficulty of selecting for such changes, and not because such mutants required the "non random gain of a highly specific function" (Gabriel et al. 1982).

Mildew able to infect equally, triadimenol treated and untreated barley crops are infrequent and may lack some aspect of fitness. Mutagenesis and recombination have so far failed to generate greater levels of resistance than found in the field, although larger samples may have revealed isolates more resistant to triadimenol. Future changes in the mildew population will depend on how readily triadimenol resistance and fitness recombine. The data presented here suggest that triadimenol resistance is controlled by many genetic factors, and mildew is likely, therefore, to respond only slowly to selection for greater resistance and fitness.

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DECREASED SENSITIVITY OF BARLEY POWDERY MILDEW ISOLATES TO TRIAZOLE AND RELATED FUNGICIDES

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ABSTRACT

Isolates of barley powdery mildew with decreased sensitivity to triadimefon showed cross-resistance to other inhibitors of sterol C-14-demethylation such as triadimenol, propiconazol, diclobutrazol, prochloraz and nuarimol. The isolates exhibited a moderate degree of resistance to these compounds. No cross-sensitivity was detected to the morpholine derivatives tridemorph and fenpropimorph. The resistant isolates were more sensitive to ethirimol than the sensitive isolate. Soil treatments with silicate ($Na_2O_7Si_3 \times n H_2O$) and potassium (KCl) retarded mildew incidence of both the sensitive and the less sensitive isolate (G 13). These treatments supported the effectiveness of triadimefon against both isolates. The competitive ability of the resistant isolate was inferior to that of the sensitive isolate and the less sensitive isolate produced fewer conidia than the sensitive one.

INTRODUCTION

The rapid increase of the use of the triazole, imidazole and pyrimidine fungicides as seed and foliar treatments for control of numerous fungal cereal diseases exerts a high selection pressure on cereal pathogens, particularly Erysiphe graminis. Several reports have demonstrated occurrence of decreased sensitivity of barley powdery mildew to triazole fungicides (Fletcher & Wolfe 1981, Laws et al. 1982, Buchenauer 1983).

This paper reports on the cross-sensitivity patterns of various isolates of barley powdery mildew obtained 1981 and 1982, the degree of resistance, and the effect of silicate and potassium and on the competitive ability of a resistant isolate with a sensitive isolate in absence of a fungicide.

MATERIALS AND METHODS

The cross-sensitivity of the different barley powdery mildew isolates was tested against various mildew fungicides. The fungitoxic agents that are systemically active were applied as root drench 7 days after sowing at the concentrations indicated: triadimefon, triadimenol, diclobutrazol, propicoanzol and nuarimol (each 0,3; 0,6; 0,9; 1,2 and 1,6 mg/pot), fenpropimorph (0,15; 0,3; 0,45; 0,6 and 0,8 mg/pot), ethirimol (0,6; 1,2; 1,8; 2,4 and 3,2 mg/pot). The compounds with inadequate systemic activity were applied as foliar spray 1 day before inoculation at the following concentrations: prochloraz and tridemorph (each 10, 25, 50 and 100 mg/l) and pyrazophos (50, 150, 300 and 500 mg/l).

Barley (Hordeum vulgare, cv. 'Gerbel') seedlings were grown in pots (diam. 9 cm) with 300 ml of a soil:sand mixture (1 : 1, v/v) at 21° C ± 5° C and 70-80 % r.h. Fertilizer (0,2 % 'Wuxal'-solution, 20 ml/pot) was applied after sowing and immediately before inoculation.

6A—7

The sensitive isolate and the various resistant isolates of powdery mildew of barley (Erysiphe graminis f.sp. hordei) were cultivated in separate greenhouse chambers.

The primary leaves of 11 day old barley seedlings were inoculated in a spore settling tower with 24 h old conidia to provide uniform inoculation. Glass slides were randomly deposited in the settling tower and the numbers of conidia were determined microscopically. If necessary, inoculation was continued until the required conidia density was obtained.

The effect of silicate and potassium either alone or in combination with triadimefon on the sensitive and the resistant isolate (G 13) of barley powdery mildew was studied. Sodium trisilicate ($Na_2O_7Si_3$ x n H_2O) (1,25 g SiO_2/kg soil) was mixed with the soil before sowing and potassium chloride (KCl) (1,5 g K_2O/kg soil) was applied as soil drench after emergence of the seedlings.

The moderately resistant isolate G 13 was used in competition experiments with the sensitive isolate. Untreated plants were inoculated with a mixture of conidia of both isolates (50 : 50 ratio). After an incubation period of 10 days conidia were used for inoculation of new untreated plants. This procedure was repeated 5 times. After each transfer changes in the resistant and the sensitive isolate were tested on untreated and triadimefon-treated (0,3; 0,6 and 1,2 mg/pot) plants.

The disease incidence was determined 8 days after inoculation by estimation of the percentage of the primary leaf area of barley seedlings that was diseased.

Conidia production was determined on detached infected primary leaves 8 days after inoculation. After determination of disease incidence and weight and shaking off the conidia sections of the primary leaves were placed in glass tubes (diam. 2,5 cm, length 25 cm) containing 5 ml dist. H_2O. The leaves were incubated (24 h at 18° C) and illuminated, then to each tube 5 ml dist. H_2O were added containing 0,1 % Tween 40. After closing, the tubes were shaken vigorously. The conidia density was determined with a microscope using a haemocytometer.

The data have been analysed statistically where possible.

RESULTS

Compared to the sensitive isolate the isolates G5, G9 and G22 exhibited a reduced sensitivity to the triazole derivatives triadimefon, triadimenol, propiconazol, diclobutrazol, the imidazole compound prochloraz, and the pyrimidine analogue nuarimol (Table 1). The factor of resistance to the various compounds of each isolate varied but the isolates were characterized by moderate degrees of resistance. No appreciable differences in the sensitivity between the sensitive isolate and the resistant pathogens were detected against the morpholine derivatives tridemorph and fenpropimorph as well as to the organophosphate derivative pyrazophos, whereas the hydroxypyrimidine derivative ethirimol controlled the resistant isolates more effectively than the sensitive isolate. The effects of negatively correlated cross-resistance to ethirimol became more pronounced when the data were based on the EC90 values: the degrees of resistance of the isolates G5, G9, G19, and G22 were 0,6, 0,7, 0,5, and 0,6, respectively.

TABLE 1:

Factors of resistance to various fungicides of some barley powdery mildew isolates. Resistance factor is expressed as ratio of the EC50 of the resistant isolate and a sensitive isolate

Fungicide	Factors of resistance of isolate G 5	G 9	G 19	G 22
Triadimefon	5	4	6	4
Triadimenol	4	3	5	5
Propiconazol	3	4	3	3
Diclobutrazol	3	4	5	3
Nuarimol	5	4	6	4
Prochloraz	4	6	7	6
Fenpropimorph	0,9	1,1	1,0	0,9
Tridemorph	0,9	1,0	1,0	1,0
Ethirimol	0,7	0,8	0,7	0,8
Pyrazophos	1,2	1,1	1,1	1,0

Soil treatment with sodium trisilicate reduced powdery mildew incidence of the sensitive and the resistant (G 13) isolate by 50 and 43%, respectively (Fig. 1). Silicate treatment not only increased the effectiveness of triadimefon against the sensitive pathogen but also against isolate G 13.

Silicate treatment combined with triadimefon treatment suppressed mildew development by 89 %.

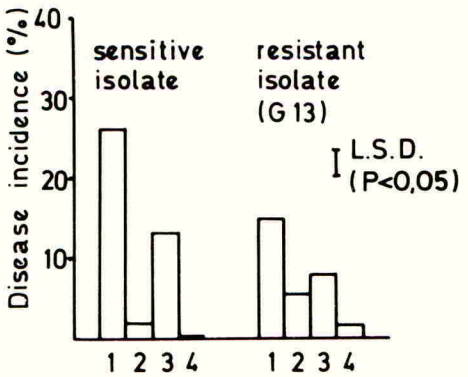

Fig. 1 Effect of soil treatment with sodium-trisilicate alone and in combination with triadimefon on incidence of barley powdery mildew

1 = Control; 2 = 0,3 mg triadimefon/pot; 3 = 1,25 g SiO_2/kg soil; 4 = 1,25 g SiO_2/kg soil + 0,3 mg triadimefon/pot

Potassium added to the soil diminished mildew incidence of both isolates on primary barley leaves by 71 % (Fig. 2). Combined appli-

cations of potassium and triadimefon tended to be more effective than single applications of either potassium or triadimefon.

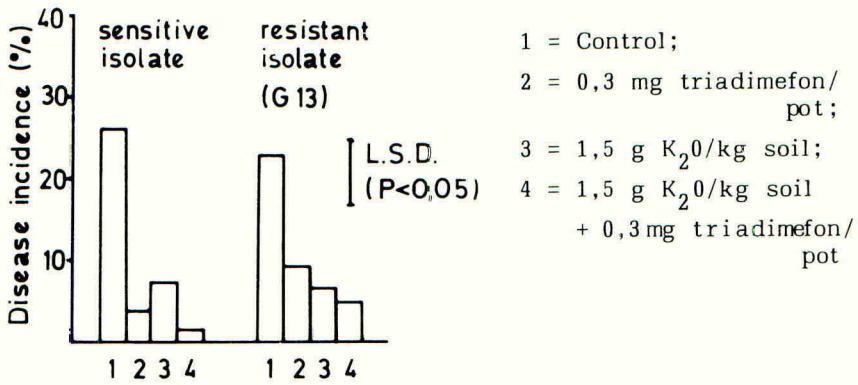

Fig. 2 Effect of soil drench with potassium alone and in combination with triadimefon on powdery mildew incidence of barley

The results of competition experiments showed that in the absence of the fungicide the portion of the resistant isolate in the population decreased after 5. passages (Fig. 3).

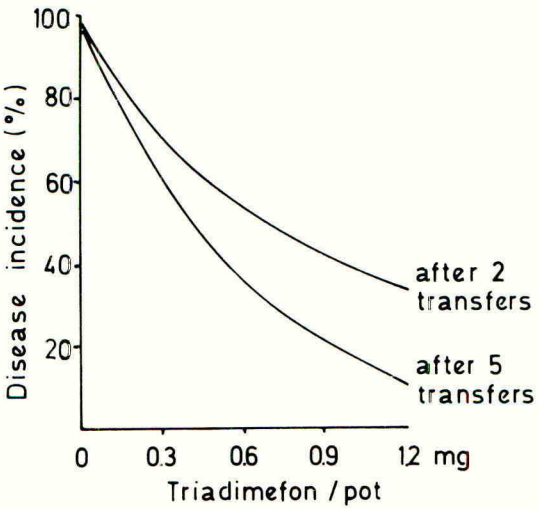

Fig. 3 Competition of a mixture of resistant (G 13) and a sensitive isolate (50 : 50) of Erysiphe graminis f.sp. hordei on primary leaves of barley seedlings after 5 transfers in absence of the fungicide

The isolate G 13 produced significantly less conidia than the sensitive isolate on barley primary leaves

DISCUSSION

Resistant isolates showed a similar pattern of cross sensitivity to the inhibitors of sterol C-14-demethylation. These results agree with those decribed by Hollomon (1982) for barley powdery mildew and those reported by Schepers (1983) for cucumber powdery mildew. The resistant mildew isolates showed no cross-resistance to the morpholine derivatives although these compounds also interfere in sterol synthesis of fungi. But unlike the triazole, imidazole, pyrimidine and piperazine derivatives they block 14/15 double bound reduction (Kerkenaar et al., 1981) and/or $\Delta 8$- $\Delta 7$ isomerization (Kato et al. 1980). Mutants of Ustilago maydis and U. avenae resistant to the inhibitors of sterol C-14-demethylation frequently show cross resistance to tridemorph and fenpropimorph. Hollomon (1982) did not detect cross-resistance between barley mildew clones less sensitive to triadimenol and the morpholine fungicides. Furthermore, the isolates with reduced sensitivity to the triazole compounds proved to be sensitive to pyrazophos. The negative cross-resistance between triadimenol and ethirimol of barley powdery mildew demonstrated by Hollomon (1982) could also be seen in our results. The barley mildew isolates are characterized by a moderate degree of reduced sensitivity. Limpert & Schwarzbach (1982) demonstrated that the resistance of barley powdery mildew isolates obtained from areas where the triazole fungicides were intensively used rarely exceeded a resistance factor of 10 and the recommended dosage of Baytan was still effective against all isolates. Schepers (1983) showed that isolates of Spaerotheca fuliginea resistant to the ergosterol synthesis inhibitors could be controlled by recommended dosages of bitertanol and fenarimol but not of triforine. On the contrary, Hollomon (1982) found wide differences in sensitivity of single pustule clones of barley powdery mildew to sterol C-14-demethylation blockers.

The disease reduction of both the sensitive and the resistant isolate by silicate treatment of barley seedlings is due to increased content of SiO_2 in the epidermal cell walls impeding the penetration of the mildew fungus in the epidermal cells. The effect of potassium during the disease development of both isolates is primarily caused by decreasing the penetration rates of barley mildew (unpublished results).

In the absence of the fungicide the isolate with reduced sensitivity showed lower competitive ability than the sensitive pathogen and there appears to exist a link between the resistance of this isolate and decreased fitness. Whether this is true of all isolates that are selected under continuous selection pressure induced by the sterol C-14-demethlylation inhibitors requires further investigation.

Pyrazophos-resistant strains of cucumber powdery mildew (Dekker and Gielink 1979) and ethirimol and tridemorph resistant isolates of barley powdery mildew (Hollomon 1975, Wamsley-Woodward et al. 1979) also showed reduced competitive abilities compared to the sensitive pathogens in absence of the fungicides.

The decrease in survival of fungicide insensitive genotypes of barley powdery mildew in north England 1982 after diminished use of triazole fungicides as reported by Wolfe et al. (1983) may be explained by the competitive disadvantage of the resistant types. The results may

contribute for development of control measures to prevent further build up or to cope with resistance of powdery mildew to sterol C-14-demethylation inhibitors.

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INVESTIGATIONS INTO THE SENSITIVITY OF WHEAT POWDERY MILDEW POPULATIONS TOWARDS FENPROPIMORPH (MONITORING PROGRAMME)

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ABSTRACT

A fenpropimorph monitoring programme in wheat was introduced in 1984. In order to establish the sensitivity of wild populations of powdery mildew to fenpropimorph, samples were collected from untreated sites at 10 different locations within the Federal Republic of Germany in May at the onset of infection. Additionally, samples were collected at 3 locations (in Schleswig-Holstein, Niedersachsen and Baden-Württemberg) from untreated, fenpropimorph or triadimenol treated experimental plots approximately 4 weeks after the last treatment. These samples were examined for their sensitivity towards fenpropimorph, triadimenol and triadimefon by different methods.

INTRODUCTION

The control of cereal powdery mildews in the Federal Republic of Germany as well as in other European countries has been primarily achieved in recent years through the application of fungicides belonging to the triazole group. As a result of this increased selection pressure, reports of reduced effectiveness of these fungicides have appeared within the last 4 years, especially with respect to barley powdery mildew (Fletcher + Wolf, 1981; Hollomon, 1982; Buchenauer, 1984). This effect has been associated with a change in the sensitivity of barley powdery mildew populations. Although complaints about lack of efficacy have been very infrequent from farmers, mildew isolates showing reduced sensitivity these fungicides have been encountered. A similar situation in wheat powdery mildew has not yet been attained but isolates can be found which have a reduced sensitivity compared with wild type isolates. A similar development has also been established in cucumber powdery mildew populations (Huggenberger et al, 1984).

Fenpropimorph, registered in England and France since 1981 and in the Federal Republic of Germany since 1983, is a new effective powdery mildew fungicide which will contribute to reducing the selection pressure. Relevant investigations have shown that cross-resistance with triazole fungicides can be ruled out, because of the difference in the mode of action of

both fungicide groups. The triazole fungicides block the C_{14}-demethylation, whereas fenpropimorph blocks the reduction of the Δ^{14} -double bond or the shift of the $\Delta^{8} \rightarrow \Delta^{7}$ double bond, i.e. later steps in ergosterol biosynthesis (Buchenauer, 1984).

There are no indications to date that the sensitivity of powdery mildew populations alters following the application of fenpropimorph (Butters et al, 1984). Glasshouse experiments carried out over a 3 year period in an attempt to induce an adaptive resistance to fenpropimorph have also produced negative results. In spite of this a monitoring programme for fenpropimorph sensitivity in populations of wheat powdery mildew was begun in 1984 and this will be continued, eventually in a more extensive form, over the next few years. In addition to the continual observations on the fenpropimorph situation, our interest lies in the establishment of the sensitivity of wild population and the variation in the range of these values and also whether the sensitivity of powdery mildew populations to triazole fungicides is influenced by the use of fenpropimorph. With this in mind the triazole fungicides triadimenol and triadimefon have been included in the investigations, acting as a form of comparative control.

MATERIAL AND METHODS

Plant material

In order to establish the sensitivity of wild populations of powdery mildew, plants showing the onset of an infection were collected during May 1984 from untreated sites at 10 different locations within the Federal Republic of Germany. These plants were potted and maintained in the glasshouse under polyethylene bags until sufficient spores had been produced for the sensitivity test.

Three locations were selected for the field monitoring programme: Kiel/Schleswig Holstein (triazoles since 1979; fenpropimorph since 1984), Hannover/Niedersachsen (triazoles since 1977; fenpropimorph since 1983) and Stuttgart/Baden-Württemberg (triazoles since 1977; fenpropimorph since 1984). Samples were collected from experimental plots (wheat cultivars: Kanzler and Götz) approximately 4 weeks after the final fungicide treatment. The treatment of the plots was as follows: untreated control, fenpropimorph (1.0 l/ha) and triadimenol (0.5 l/ha), in Stuttgart the additional treatment triadimefon (0.5 l/ha) was also sampled. Wheat plants were sampled from the plots and treated as described above. The sensitivity tests were conducted with spore material collected from these plants where this was possible, however, where the original level of infection was so low that insufficient spores were produced for the test, then an intermediate passage on young wheat plants (cv. Kanzler) was made.

Sensitivity test

Wheat seedlings (cv. Kanzler) at the first leaf growth stage were used in the test. These were sprayed to run-off with a range of concentrations (0-500 ppm a.i.) of fenpropimorph, triadimenol and triadimefon 24 h before inoculation. Two different methods were employed:

1) Segments of the first leaf (5 leaves for each treatment and concentration) from treated and untreated control plants were inserted into an agar medium containing 10 ppm benzimidazole in plastic boxes (1 box/concentration and fungicide) and inoculated by spraying with a spore suspension in oil (Perfluortributylamine, Fluka AG; 2 mg spores/ml oil).
2) Entire plants (1 pot of 10 plants for each treatment) were inoculated by dusting the plants with spores.

All the samples collected were tested using both methods. In order to avoid vapour contaminations pots were put into plastic cylinders with an open top directly after spraying of the plants. When method 2 was used plants remained in these cylinders until evaluation.

The plastic boxes and plant pots were then placed in a climate chamber in which a temperature of 18 °C was maintained and a light source of 8000 lux was provided 14 h/day.

The results were recorded after 8-10 days incubation by either counting the number of powdery mildew pustules on the first leaf segments or counting the number of pustules on 5 first leaves in each pot.

ED_{50}- and ED_{98}-values were calculated from dose-response curves.

RESULTS AND DISCUSSION

Under the conditions used for the test, both test methods produced comparable results. Because of this the results obtained were treated as replicates; the figures in the tables represent a mean value from both test methods.

The ED_{50}- and ED_{98}-values for fenpropimorph of 7 wild populations are listed in Table 1. Powdery mildew did not develop on 3 further samples in this series. Although the ED_{50}-values of all 7 samples lie relatively close together, the range in the ED_{98}-values (3-15 ppm a.i.) is relatively wide. The so-called "laboratory isolate" was included in the test as a reference isolate on account of its guaranteed sensitivity. The ED_{50}- and ED_{98}-values for triadimenol and triadimefon were also established for this isolate: triadimenol 1,5 and 4,0 ppm a.i., triadimefon 1,6 and 8,0 ppm a.i.,respectively.

TABLE 1

Sensitivity of wild populations of wheat powdery mildew towards fenpropimorph

Location	Variety	Fenpropimorph ED_{50} (ppm a.i.)	Fenpropimorph ED_{98} (ppm a.i.)
Hannover	Kanzler	1,8	13
Köln	Okapi	1,8	5
Oldenburg	Kanzler	3,9	15
Münster	Kanzler	2,8	11
Erlangen	Kanzler	1,5	3
Böhl	Kanzler	2,0	8
München	Kanzler	1,6	7
"Laboratory Isolate"		2,9	11

The results of the investigations made at the three field locations Kiel, Hannover and Stuttgart are presented in Table 2. In addition to the ED98-values for the powdery mildew populations found in the plots, the disease assessments made at the time the samples were collected are also given.

TABLE 2

Sensitivity of several wheat powdery mildew populations in trial plots towards fenpropimorph, triadimenol and triadimefon (powdery mildew assessment and sampling 4-5 weeks after the final fungicide application).

Treatment of trial plots	powdery mildew assessment of plots % leaf area infected	ED_{98} of powdery mildew populations (ppm a.i.) for 3 fungicides I*	II	III
Location Kiel:				
Untreated control	40	6	31	77
Triadimenol	20	9	33	55
Fenpropimorph	15	6	33	59
Location Hannover:				
Untreated control	14	15	14	39
Triadimenol	4	14	23	40
Fenpropimorph	3	11	14	41
Location Stuttgart:				
Untreated control	30	-	-	-
Triadimenol	12	6	13	34
Triadimefon	15	4	15	48
Fenpropimorph	6	4	18	41

*I = fenpropimorph; II = triadimenol; III = triadimefon

The influence which a treatment can have on the reaction of a powdery mildew population is indicated in the samples collected in Kiel. The sensitivity of the population towards triadimefon was increased following treatment with fenpropimorph and/or triadimenol. It remains to be seen in the following years whether this is a general development or was achieved in this case by chance.

As was to be expected from literature data on the sensitivity of mildew populations (Buchenauer, 1984) the highest ED_{98}-values were found for triadimefon. The triadimenol values are clearly lower than those for triadimefon and lie sometimes, as can be seen in the values from Hannover at the same level as fenpropimorph. Triadimenol as well as triadimefon values found at all 3 locations are greater than those established for the sensitive laboratory isolate.

The fenpropimorph values from all 3 locations were found to be independent of treatment and lie in the range of values found with the wild populations.

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RESISTANCE TO INHIBITORS OF STEROL BIOSYNTHESIS IN CUCUMBER POWDERY MILDEW

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ABSTRACT

Fungicides which inhibit ergosterol biosynthesis (EBIs) have been used almost exclusively since 1981 for control of cucumber powdery mildew (Sphaerotheca fuliginea) in glasshouse-grown cucumbers in the Netherlands. This intensive use caused a gradual decrease in sensitivity of S. fuliginea to EBIs. Performance of triforine declined to such an extent, that growers do not apply it anymore in case of high disease pressure. A change to shorter spray intervals has up till now been sufficient to achieve proper control by bitertanol, fenarimol and imazalil.

INTRODUCTION

During the past four years control of cucumber powdery mildew (Sphaerotheca fuliginea) in the Netherlands has been carried out almost exclusively with the ergosterol biosynthesis inhibitors (EBIs) bitertanol, fenarimol, imazalil and triforine. Such a practice may lead to the development of resistance, especially because this pathogen seems to be prone to resistance (Schepers 1983, Huggenberger *et al.* 1984).

The development of resistance to EBIs was followed in a monitoring study from 1981 to 1984.

MATERIALS AND METHODS

The first survey in October 1981 covered 50 glasshouses distributed over the main cucumber-growing areas of the Netherlands (Schepers 1984a). In 1982, 1983 and 1984, isolates were obtained from 13 glasshouses. In 1982 and 1983 the mildew population in these glasshouses was sampled three times in one growing season namely before the first fungicide treatment (January to May), in July, and in October. In 1984, isolates were collected up to July.

For each isolate, 5 to 10 infected leaves were collected from a small area (1 to 2 m^2) in a glasshouse. The isolates were transferred to mildew-free cucumber plants (cv. Lange Gele Tros) and subcultured once or twice, until sufficient conidia were available for testing. The sensitivity to EBIs of the isolates was determined in leaf disc and foliar spray tests (Schepers 1984b). The sensitivity to EBIs of the glasshouse isolates was compared with that of reference isolates. The reference isolates have never been in contact with fungicides and are regarded to have a wild-type sensitivity.

RESULTS AND DISCUSSION

Isolates collected in 1981 had a lower sensitivity to fenarimol than the reference isolates (Table 1). This may be due to the use of triforine and imazalil since 1972 and 1977, respectively. After the introduction of fenarimol in 1981, the use of EBIs increased rapidly. This may explain the increase in number of isolates with a decreased sensitivity to fenarimol (Table 1) and imazalil in 1982 and 1983. There are indications that isolates collected after breaks in the growing season (winter) displayed a slightly higher sensitivity than those collected before the break. In 1982 and 1983 a change to shorter spray intervals was sufficient to achieve proper mildew control with fenarimol

and imazalil. However, triforine often proved to be ineffective. This was confirmed in foliar spray tests (Schepers 1983). The first results of 1984 show that the level of resistance is not higher than in 1983, although the decrease in number of isolates with EC_{50} values between 4 and 8×10^{-8}M continued. A better disease control strategy and the late start of the mildew epidemic in 1984 might explain this delay in the development of resistance. This observation is reassuring but the possibility that the sensitivity to EBIs of the glasshouse isolates will continue to decrease cannot be excluded.

TABLE 1

EC_{50} values (10^{-8}M) of fenarimol for inhibition of mycelial growth in leaf disc tests of Sphaerotheca fuliginea isolates collected in 1981 to 1984 in the Netherlands and of reference isolates (ref.)

Year	Number of isolates in each EC_{50} category						
	0 - 2	2 - 4	4 - 8	8 - 16	16 - 32	32 - 64	>64
1981	0	1	35	20	41	0	0
1982	1	0	18	19	35	2	0
1983	0	0	6	20	42	45	13
1984	0	0	2	21	58	16	0
ref.	51	4	1	0	0	0	0

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EFFECTIVENESS OF FOSETYL AL AGAINST STRAINS OF PLASMOPARA VITICOLA AND PHYTOPHTHORA INFESTANS THAT HAVE DEVELOPED RESISTANCE TO ANILIDE FUNGICIDES

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ABSTRACT

It has been demonstrated in studies where systemic fungicides were in contact with leaf disks of potato or vine that metalaxyl resistant strains of Phytophthora infestans or of Plasmopara viticola had developed no resistance to fosetyl Al. However, the efficacy of fosetyl Al against resistant strains of P. viticola may be affected by the presence of low concentrations of metalaxyl brought onto the disks as a result of "vapour" action. This phenomenon is probably due to the stimulation of the resistant strains under certain conditions by metalaxyl. It probably accounts for an earlier report of cross resistance between metalaxyl and fosetyl Al.

INTRODUCTION

Soon after the launching of fungicides from the anilide class of compounds (N-phenyl amides also referred to as acylalanines) resistant strains were observed among the populations of various oomycete species (Staub and Sozzi 1981, Davidse 1982). Whereas in some cases, these fungicides can be combined or used alternately with compounds from other families as an anti-resistance strategy (Staub and Sozzi 1983, Levy et al 1983), they may sometimes have to be abandoned when heavy resistance is met. This necessitates full awareness of the cross resistance risks between anilides and other fungicides for possible use as replacements, partners, or in alternating treatment programmes. While a large number of laboratory tests, mostly unpublished, carried out throughout the world come to the conclusion that there is no cross-resistance between anilides (metalaxyl, ofurace, benalaxyl, cyprofuram, oxadixyl) and other oomycete fungicides, a recent publication (Cohen and Samoucha 1984) states Phytophthora infestans and Pseudoperonospora cubensis as being cross-resistant to metalaxyl, propamocarb and fosetyl Al. These results are all the more surprising as all these fungicides have a basically different mode of action. The purpose of this paper is to shed light on the purported cross-resistance to fosetyl Al in the case of P. infestans and P. viticola , two downy mildew pathogens particularly important in France (Leroux and Clerjeau 1984).

MATERIAL AND METHODS

The same method was used in all tests: artificially infected leaf disks were placed in contact with the fungicides in Petri dishes.

Fungicides

Aqueous suspensions of metalaxyl and fosetyl Al were obtained using 'Ridomil' 25% and 'Aliette' 80% respectively.

Fungi

- P. infestans: 4 strains, cultured in a V8 medium, were used, strain L1, susceptible to metalaxyl, strain L2, partly resistant (showing 50% inhibition at 10 ppm) and strains R29 and R31, both resistant and uninhibited at a concentration of 100 ppm, received from Dr. Davidse.

- P. viticola: To investigate possible cross-resistance between metalaxyl and fosetyl Al, we used two reference strains from the laboratory; one susceptible strain whose sporulation on vine leaf disks was non-existant at 1 ppm metalaxyl and the other resistant (resistance factor > 100).

The study as to how metalaxyl affected sporulation in resistant strains was carried out using 51 populations of P. viticola originating from sporulating lesions collected during 1983 in various vineyards treated with anilide-based fungicides (metalaxyl or ofurace).

Treatments and artificial inoculations

- P. infestans: Potato leaf disks (var. Bintje), 36 mm in diameter, placed in Petri dishes on a 15 ml solution of either metalaxyl or fosetyl Al were inoculated on the underside by placing a mycelial explant in a small wound on the main rib. Thirty disks were used for each fungicide concentration. Following a 5-day incubation period in a microphytotron, at 20°C, with constant lighting conditions the following 3 factors were assessed: the percentage of disks totally or partly attacked, the surface area of infected tissue and the number of sporocysts produced (counted in a heamocytometer).

- P. viticola: Vine leaf disks (var. Muscadelle), 18 mm in diameter, were placed in Petri dishes on a filter paper impregnated with 7 ml of fungicide suspension and later inoculated with 3 drops of 20 μl of a sporocyst suspension containing 2.10^4 spores/ml. Each treatment included 30 inoculations on 10 disks. After a 7-day incubation period, under fluorescent lighting operating 16 hrs/day, the number of sporulated spots and level of sporulation was scored. A mark between 0 and 4 was allotted to each inoculation point according to the abundance of visible sporulation.

In order to appraise the consequences of the "vapour effects" of metalaxyl, the dishes containing the disks to be inoculated were placed into larger, closed dishes (13 cm in diameter) containing 50 ml metalaxyl at 100 ppm. Under these conditions, and as a result of co-distillation with water vapour, metalaxyl was redistributed at a low concentration into the dishes containing the disks as though carried in vapour (Clerjeau et al 1981).

All 51 resistant populations were inoculated immediately on receipt at the laboratory, the medium and inoculum concentration used being identical whether the disks had been treated with metalaxyl or not.

RESULTS

Susceptibility of metalaxyl resistant strains of P.infestans to fosetyl Al

It can be seen from table 1 that although the efficacy of fosetyl Al against P. infestans is rather low, it is not significantly different between metalaxyl susceptible or resistant strains, whatever assessment method is used.

TABLE 1
Susceptibility to fosetyl Al of 4 strains of P. infestans sensitive (S), partly resistant (r) or resistant (R) to metalaxyl on potato leaf disks

Strains (Ref.)	Treatments	% disks attacked totally	% disks attacked partly	Infected surface area (cm2)	%inhibition sporulation/ control
S (L1)	Control	90 %	10 %	277.3	
	Metalaxyl 20 ppm	0 %	96.7 %*	16.8	100 %
	Fosetyl Al 500 ppm	60 %	36.7 %	217.7	59.6 %
r (L2)	Control	90 %	6.7 %	275.2	
	Metalaxyl 20 ppm	13.3 %	80 %	67.7	73.5 %
	Fosetyl Al 500 ppm	40 %	36.7 %	141.2	25 %
(R29)	Control	90 %	10 %	276.4	
	Metalaxyl 20 ppm	100 %	0 %	305.2	38.9 %
R	Fosetyl Al 500 ppm	40 %	56.7 %	171.3	50 %
(R31)	Control	100 %	0 %	305.2	
	Metalaxyl 20 ppm	100 %	0 %	305.2	0.05%
	Fosetyl Al 500 ppm	33.3 %	66.7 %	156.9	68.9 %

(*) Very slight necrosis showing no further evolution during test

The fact that in the presence of fosetyl Al colonized areas of leaf surface are smaller for strains resistant to metalaxyl than with susceptible ones should not be interpreted as a case of negative cross resistance. It is caused by the lower growth rate in vitro of the strains L2, R29 and R31.

Susceptibility to fosetyl Al of metalaxyl resistant strains of P. viticola

In field or greenhouse fungicide tests metalaxyl can act from a distance, "vapour effect", by co-distillation with water vapour (Clerjeau et al 1981). The resulting effects are similar to those that would be produced by the treatment, at a low dose, of neighbouring plots or plants and may interfere with the interpretation of the behaviour of fungicides. For this reason the efficacy of fosetyl Al against sensitive and resistant strains was examined under two different conditions, i.e. with and without metalaxyl vapour effects. It can be seen from the results summarized in Table 2 that:

- without vapour effect from metalaxyl, the metalaxyl-resistant and the metalaxyl-susceptible strains showed similar susceptibility to fosetyl Al. No cross-resistance between these two fungicides was observed.

- when disks are placed in the presence of the "vapours" of metalaxyl which give a degree of protection against the sensitive strain which averages 90%, the disks treated with the highest concentration (600 ppm) of fosetyl Al, as previously, are totally protected against the sensitive or resistant strains.

TABLE 2

Levels of attack of P. viticola strains susceptible (S) and resistant (R) to metalaxyl, obtained on vine leaf disks treated with fosetyl Al or metalaxyl, with or without the presence of metalaxyl "vapours"

Treatment of inoculated disks	With (+) or without (-) metalaxyl in the atmosphere	% sporulating lesions*		Sporulation rate**	
		Strain S	Strain R	Strain S	Strain R
	Inoculated leaf disks				
Control	(-)	100 %	100 %	87 %	100 %
Metalaxyl 5 ppm		0 %	100 %	0 %	100 %
Fosetyl Al 200 ppm		50 %	20 %	37 %	12 %
Fosetyl Al 600 ppm	Water	0 %	0 %	0 %	0 %
Control	(+)	10 %	100 %	10 %	100 %
Metalaxyl 5 ppm		0 %	100 %	0 %	100 %
Fosetyl Al 200 ppm		0 %	70 %	0 %	30 %
Fosetyl Al 600 ppm	Metalaxyl 100 ppm	0 %	0 %	0 %	0 %

(*) percent of control (without any fungicide)

(**) calculated by giving sporulating lesions marks ranging from 0 to 4 , and expressing the results as a percentage of the maximum possible score

At a concentration of 200 ppm fosetyl Al, which is only partly effective against P. viticola (50% of sporulated disks compared with control), the additional effect of metalaxyl is shown by complete

protection against the sensitive strains and lesser protection against the resistant strains. The infection in this case could be misinterpreted as cross-resistance if the additional "vapour" action of metalaxyl is not taken into account.

Although the additive affect of metalaxyl and fosetyl Al could reasonably be expected, the reduced activity of fosetyl Al against the resistant strains in the presence of small amounts of metalaxyl is most surprising. However, it was apparent in several replicate experiments. The results of Figure 1 provide an explanatory hypothesis.

These results obtained from 49 resistant populations, reveal that, under poor inoculation conditions (linked to the medium, the host or the inoculum), resulting in low levels of attack (under 20-30%), the addition of metalaxyl enhances the development of the disease.

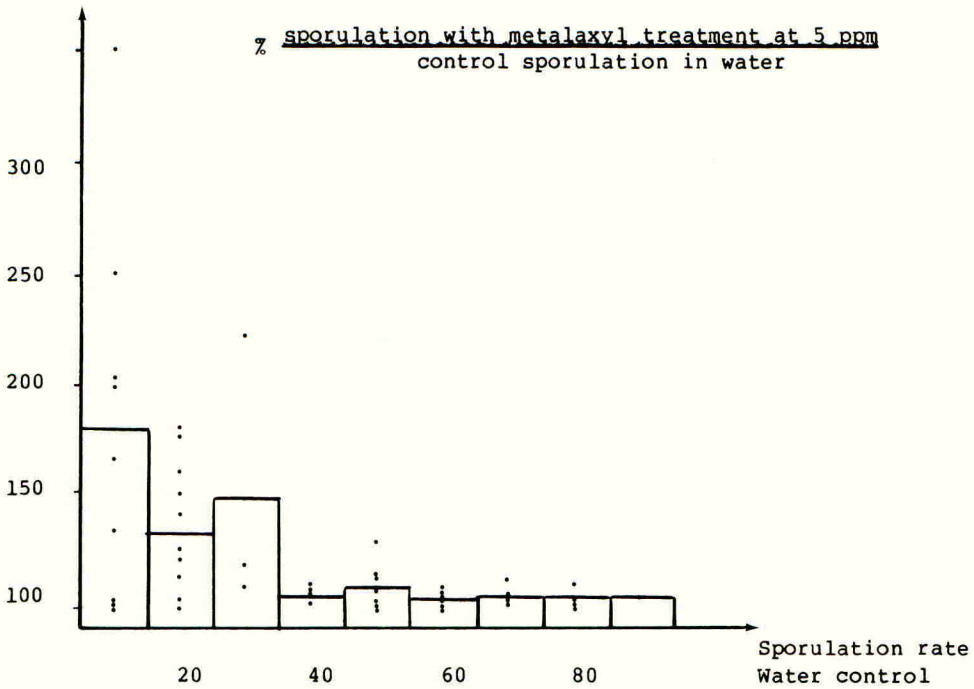

FIGURE 1
Influence of metalaxyl (at 5 ppm) on the sporulation of 49 resistant populations of P. viticola on leaf disks

It may be postulated from these data that in tests including metalaxyl, the poorer efficacy of fosetyl Al against resistant strains is the indirect effect of metalaxyl causing attacks to be more virulent.

CONCLUSION

These results clearly show that for strains of P. viticola having developed resistance to anilides, fosetyl Al may prove an effective alternative. This was in fact duly observed in many tests carried out in vineyards against resistant populations. Although there is no cross-resistance between these two fungicides, it has to be noted, however, that when exceptional conditions are met, i.e. when plants are treated with an only partly effective dose of fosetyl Al and are moreover in contact with metalaxyl at a low dose e.g. as "vapours", the control of susceptible strains is increased and that of resistant ones reduced. Such poorer efficacy of fosetyl Al against resistant strains is not to be interpreted as cross-resistance. To account for it, the following hypothesis may be advanced: a complete protection against susceptible strains is ensured by the combined effects of the two fungicides, whereas only fosetyl Al shows any effect against resistant strains. It brings the inoculum rate down to a low level (12% sporulation rate). Under these extreme conditions for the development of the disease, metalaxyl may stimulate the pathogen (30% sporulation rate) as shown in figure 1. Our knowledge to date prevents us from establishing whether this antagonistic process between fosetyl Al and metalaxyl affecting resistant strains of P. viticola may be observed in other host-phycomycete relationships or whether it is likely to result in practical consequences in the field when fungicides are used in combination or one after the other.

The results we obtained with P. infestans disagree with those of Cohen and Samoucha (1984) stating metalaxyl and fosetyl Al to be cross-resistant. Under the conditions selected by these two authors, the plants treated with each of these fungicides were located in the same enclosed space, in a greenhouse. Considering the phenomena demonstrated with P. viticola, one may wonder whether metalaxyl did not act from a distance by "vapour" on the plants treated with fosetyl Al and contaminated by susceptible or resistant strains.

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RESISTANCE OF ISOLATES OF PHYTOPHTHORA INFESTANS TO FUNGICIDES

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ABSTRACT

The sensitivities of seven field isolates of Phytophthora infestans to eight fungicides, all used to control Oomycetes, have been measured both in vitro and in vivo. Five of the isolates were resistant in vivo to metalaxyl and also showed resistance to other fungicides from the 'acylalanine' group, namely ofurace, SAN 371 F and cyprofuram. Levels of resistance to cyprofuram were consistently lower. Sensitivity in vitro to the acylalanines followed the same pattern. Resistance to metalaxyl did not confer cross-resistance to four unrelated fungicides, etridiazole, propamocarb, fosetyl-Al and cymoxanil. One acylalanine-sensitive isolate showed exceptionally high sensitivity in vivo (but not in vitro) to the latter three fungicides but this was associated with variable pathogenicity to leaf discs.

INTRODUCTION

Acylalanine fungicides, which show systemic and curative activity against potato blight (Phytophthora infestans), represent a major advance in the control of this disease (see Schwinn 1983). Metalaxyl and, to a lesser extent, ofurace, have been used for several years for blight control; several recently introduced fungicides of this type, including cyprofuram (Baumert & Buschhaus 1982) and SAN 371 F (Gisi et al. 1983), are also active against P. infestans. The term 'acylalanine' is commonly applied to all these compounds although ofurace and cyprofuram are butyrolactones and SAN 371 F (2-methoxy-N-(2-oxo-1,3-oxazolidin-3-yl)-acet-2',6'-xylidine) is an oxazolidinone.

During 1980 the emergence of resistant forms of P. infestans caused a breakdown in control of potato blight with metalaxyl in Holland (Davidse et al. 1981) and Eire (Dowley & O'Sullivan 1981). Metalaxyl-resistant forms of blight have also been detected in N. Ireland (Cooke 1981), England (Carter et al. 1982) and Israel (Cohen & Reuveni 1983). Cross-resistance in Oomycetes has been demonstrated between metalaxyl and both ofurace (e.g. Davidse et al. 1981) and cyprofuram (Katan 1982, Cohen & Samoucha 1984). Cross-resistance between metalaxyl and SAN 371 F has also been reported (Gisi et al. 1983) but resistance to the latter was at a lower level. Wider cross-resistance involving chemically unrelated fungicides has recently been reported (Cohen & Samoucha 1984).

In this paper we report upon the sensitivity of isolates of P. infestans, obtained from potato crops in the UK, to four 'acylalanines' (metalaxyl, ofurace, SAN 371 F and cyprofuram) and four unrelated fungicides (etridiazole, propamocarb, fosetyl-Al and cymoxanil), all of which show systemic activity against Oomycetes (Schwinn 1983).

MATERIALS AND METHODS

Chemicals

All compounds were tested as unformulated technical grade materials.

Metalaxyl, ofurace, cyprofuram, propamocarb, cymoxanil and fosetyl-Al were supplied as such whilst SAN 371 F and etridiazole were extracted from commercial formulations.

Phytophthora isolates

Isolates A to F were obtained in 1982 from blighted potato leaves taken from crops growing in S.W. England. Isolate G is a single zoospore isolate from F. All isolates were maintained by continuous transfers onV-8 juice agar and/or potato leaf tissue.

Sensitivity testing

In vitro

The test fungicides were incorporated, after autoclaving, into plates of rye seed extract agar and each plate inoculated at the centre with a 5 mm plug cut from the periphery of a young mycelial colony of P. infestans. Colony diameters were measured after 5 - 7 days incubation at 21°C.

In vivo

Leaf discs, inoculated at their centre with a single 10 µl droplet of a mixed suspension of sporangia and zoospores, (initially containing c. 1000 sporangia), were floated upon solutions of the fungicides. After 7 days incubation in a growth cabinet disease development was recorded using a scale from 0 (no visible symptoms) to 5 (discs completely covered with sporulating blight) (see Carter et al. 1982 for details).

RESULTS

Acylalanines

In leaf disc tests (see Table 1) two isolates (A & B) showed high sensitivity to metalaxyl whilst the remainder (C to G) were resistant. These metalaxyl-resistant isolates all showed a similar level of resistance to both ofurace and SAN 371 F but a variable, and consistently lower, level of resistance to cyprofuram. Isolates sensitive to acylalanines (and other fungicides) always gave a grade 1 reaction (necrotic flecking under the inoculum droplet) rather than grade 0 (no visible symptoms).

Sensitivity to acylalanines in agar growth tests showed a similar pattern to that shown in vivo. Thus growth of isolates A & B was completely inhibited by 1 µg/ml of all four fungicides. Growth of the other five isolates was virtually unaffected by 100 µg/ml of ofurace and SAN 371 F; metalaxyl at this concentration decreased growth of these isolates by from 4 - 42% whilst cyprofuram was more effective causing from 25 - 66% decrease in colony size.

Other fungicides

Sensitivity in vivo to the non-acylalanines is given in Table 2. All isolates showed a uniformly moderate sensitivity to etridiazole. Sensitivity was apparently lower to propamocarb and fosetyl-Al and varied between isolates. Variation in the response of different isolates was even more marked with cymoxanil. Isolate B was by far the most sensitive to all three fungicides and also gave rather erratic levels of infection on untreated leaf discs.

Sensitivity in vitro to fosetyl-Al and propamocarb (see Table 3) varied between isolates but was rarely high. At 100 µg/ml fosetyl-Al caused 0 - 34% decrease in colony diameter and propamocarb 39 - 100%.

Etridiazole was fully effective against most isolates at 10 µg/ml whilst cymoxanil, the most active compound, was fully inhibitory at 1 µg/ml against all isolates.

TABLE 1

Response[1] *in vivo* in a leaf disc, of isolates of *Phytophthora infestans* to acylalanine-type fungicides

Fungicide	Conc. (µg/ml)	Isolate A	B	C	D	E	F	G
None	-	5	2-5	5	5	5	5	4-5
Metalaxyl	1000		1	2-3	1-2	2-3	1-2	1-2
	100	1	1	4	5	5	3	3-4
	1	1	1	5				
Ofurace	500[2]		1	5	5	5	5	5
	100	1	1	5	5	5	5	4-5
	1	1	1					
SAN 371 F	1000		1	5	5	5	5	4-5
	500	1	1	5	5	5	5	4-5
	1	1	1					
Cyprofuram	500		1	1	1	1	1	1
	100	1	1	3-4	3-4	2-3	1	1-2
	10	1	1	5	5	5	5	4-5
	1	1	1					

[1] Values are estimates of disease development on inoculated leaf discs (scale 0-5).

[2] Out of solution.

DISCUSSION

A specific change in a fungus can render it resistant to all fungicides sharing the same mode of action; this phenomenon is generally called cross-resistance (see Georgopoulos 1982). An apparent cross-resistance to unrelated fungicides could also occur through either a less specific change (such as a reduction in membrane permeability or pathogenicity) or the presence of more than one resistance gene in the same organism (multiple resistance). The mechanism of resistance to metalaxyl is not yet established but it is neither reduced uptake nor increased metabolic detoxification (Fisher & Hayes 1984).

The fungitoxic action of metalaxyl, and probably also ofurace, involves partial inhibition of nucleic acid synthesis (see Fisher & Hayes 1984). The high level of cross-resistance shown in these studies between metalaxyl, ofurace and SAN 371 F strongly suggests that they share the same mode of action but the consistently lower degree of resistance shown to cyprofuram could indicate that this compound has a different, or an

TABLE 2

Response[1] *in vivo* of isolates of *Phytophthora infestans* to four, non-acylalanine fungicides

Fungicide	Conc. (µg/ml)	Isolate A	B	C	D	E	F	G
None	-	5	4-5	5	4-5	5	5	5
Etridiazole	100	1	1		1	1	1	1
	10	1	1	1	1	1	1	1
	1	5	2-3	5	3-4	4-5	3-4	5
Propamocarb	100	4-5	1	2	3-4	1	1-2	1
	10	5	1	4-5	4-5	4-5	3-4	5
	1	5	2-4	4-5	4-5	4-5	2-3	5
Fosetyl-Al	100	1	1	1	1	1	1	1
	10	5	1	3-4	2-3	3	3-4	3-4
	1	5	4-5	4-5	4-5	4-5	3-4	5
Cymoxanil	100	1	1	1	1	1	1	1
	10	4-5	1	4-5	2	4-5	1	1
	1	5	1	5	4-5	4-5	3-4	5

[1]Values are estimates of disease development on inoculated leaf discs (scale 0-5).

TABLE 3

Response[1] in mycelial growth tests, of isolates of *Phytophthora infestans* to four, non-acylalanine fungicides

Fungicide	Conc. (µg/ml)	Isolate A	B	C	D	E	F	G
Etridiazole	10	0	0	0	0	0	28	0
	1	19	42	43	48	53	77	53
	0.1	98	99	100	100	100	100	100
Cymoxanil	10	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0
	0.1	21	72	68	72	69	68	66
Propamocarb	100	61	59	33	30	0	45	44
Fosetyl-Al	100		99	67	67	66	79	100

[1]Values are colony diameter as percentage of control (untreated).

additional, toxic mechanism. It has been suggested (e.g. Bruin & Edgington 1982) that activity of metalaxyl in vivo is not due solely to direct fungitoxic action but the close correlation found by us between relative sensitivity to acylalanines in vitro and in vivo points to a predominantly direct fungitoxic action within leaf tissues. Other workers (Coffey & Young 1984) however, have found discrepant behaviour in vitro and in vivo with metalaxyl-resistant isolates of P. infestans.

Etridiazole, propamocarb, fosetyl-Al and cymoxanil are thought to have a different mode of action both from acylalanines and from one another (see Langcake et al. 1983). Therefore it seems unlikely that cross-resistance between metalaxyl and these fungicides will occur and there is some evidence to support this view (e.g. Davidse et al. 1981, Cooke 1981). Recently, however, there has been a report (Cohen & Samoucha 1984) of metalaxyl-resistant strains of P. infestans being insensitive in vivo to propamocarb and fosetyl-Al.

None of the isolates examined by us showed evidence of reduced sensitivity to etridiazole. If the high sensitivity of isolate B in vivo to propamocarb, fosetyl-Al and cymoxanil is taken as the normal 'wild-type' level, then all other isolates (including isolate A which is highly sensitive to acylalanines) must be considered somewhat resistant to all three fungicides. However, isolate B is probably atypical, a view supported by its variable pathogenicity and the fact that it is not especially sensitive to these fungicides in vitro. Differences in sensitivity to propamocarb and fosetyl-Al shown by other isolates probably are no more than 'normal' variation among different isolates of any fungus; they do not correlate with sensitivity to acylalanines. Differences in sensitivity in vivo to cymoxanil are more pronounced but they are not reflected in vitro, where all isolates behaved similarly. More data is needed to establish clearly the basic range of sensitivities to this fungicide in vivo.

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FUNGICIDE RESISTANT STRAINS OF *VENTURIA INAEQUALIS* IN KASHMIR- A PREDICTION

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ABSTRACT

Monitoring of fungicide resistant strains of the apple scab pathogen (*Venturia inaequalis*) from forty orchards in different locations in Kashmir Valley (J & K State) was carried out just before harvest during 1981 and 1982 seasons.

Disease control in some of these orchards where mancozeb or carbendazim based fungicides had been exclusive ly applied during the past was found inadequate. On the basis of resistant factor, emergence of resistant strains of the pathogen against these fungicides in Kashmir has been demonstrated.

INTRODUCTION

Apple scab (*Venturia inaequalis*) is one of the five main pest problems of national importance in India (Anon., 1974) and is causing severe losses to the fruit industry in two leading apple growing states- Jammu & Kashmir and Himachal Pradesh (Rao 1983).

In Kashmir Valley, the disease first assumed epiphytotic form in 1973 involving all the commercially important cultivars (Puttoo, 1974; Joshi *et al*, 1975; Malik *et al*, 1976; Puttoo *et al*, 1976). Since then the disease has become endemic in all the important apple growing belts covering an area of 60,000 ha The ravages of sacb have, to some extent been checked by repeat ed application of fungicides during the season. Exclusive and extensive application of mancozeb from 1972 onwards in some orchards gave adequate control till 1980 but in subsequent seasons the level of control showed a decline. Emergence of resistant strains of the pathogen in such orchards was suspected.

Studies were carried out during 1981 and 1982 season to monitor the resistant strains in 40 problem orchards located in different parts of the Kashmir Valley.

MATERIALS AND METHODS

Selection of orchards

Forty orchards were identified on the basis of the awareness the growers had towards the plant protection coupled with their observations on the ineffectiveness of the fungicides used. The information about the number, timing and sequenceof fungicide applications and the fungicide used in the past was collected by devising a suitable questionnaire. Percent disease control on leaves in each orchard was recorded after the completion of the sprays.

Mycelial sensitivity

Poisoned food technique adopted for the toximetric studies by Carpenter (1942) was followed. A series of concentrations of each of the test fungicide formulation was prepared by dilution technique. Equal volumes of double strength fungicide suspension and malt extract agar medium were mixed thoroughly and poured in culture tubes aseptically. The slants were inoculated with mycelial bits from all the isolates separately and incubated at 18 ± 2 C for 21 days. Growth of the colony was the criteria adopted for observations.

Stability of resistance

The stability of resistance was studied by growing the isolates on fungicide-free medium by subculturing four times (one every third week). Each subculture was considered to be a generation (Kumar and Sastry, 1979). The fifth generation was again grown on fungicide amended medium of the concentration that had earlier permitted mycelial growth. Isolates that grew on the amended media were rated as stable while others were considered unstable.

Pathogenicity

One year old budded Cv.Red Delicious apple saplings grown in clay pots were used for pathogenicity tests. A conidial suspension (50,000 to 70,000 conidia/ml) was prepared from each isolate and sprayed on the plants with an atomizer. After the droplets had dried saplings were given an artificial infection period for 48 hours at 16-20 C, at apprx. 100% relative humidity. The saplings were incubated for 10 days in a glass house for the symptoms to develop.

RESULTS

Surveys reveal that control practices adopted in orchards vary in different localities (Table 1). Out of forty orchards 7 received mancozeb exclusively while only one received carbendazim during 1981. During 1982, the exclusive usage of mancozeb was replced by inclusion of other fungicides in the schedule. Mancozeb has been a principal fungicide used in the past. The percent disease control ranged from 41.20 to 74.65 in orchards that received mancozeb exclusively. The level of disease control fell from 95.27 to 77.16 percent in the orchards that received exclusively carbendazim during 1981 and 1982.

Isolates exhibited differential response during mycelial growth tests against test fungicides (Table 2). The magnitude of response in six isolates was 1.5 to 2.5 fold against mancozeb and 3 - 14 fold against carbendazim in three isolates. The isolates are grouped as:

1. Resistant to mancozeb only	Isolates from Chitragam and Arihal
2. Resistant to carbendazim only	Isolates from Kriri, Imam saheb
3. Combined resistance to mancozeb and carbendazim	Isolate from Seer Jagir

TABLE 1
Impact of fungicide spraying operations against apple scab (Venturia inaequalis) on leaves in selected problem orchards in Kashmir Valley

Location	Number of sprays applied		Sequence of fungicides*		Percent disease control		Principal fungicide
	1981	1982	1981	1982	1981	1982	
Seer Jagir	5	4	e	a	72.56	59.42	mancozeb
Chitragam	6	5	e	a	74.65	84.29	mancozeb
Mattan Karewa	4	2	e	e	65.54	17.07	mancozeb
Thagiwara	3	3	e	a	54.14	51.21	mancozeb
Kriri	3	3	e	e	95.77	77.16	carbendazim
Imamsaheb	6	4	a	a	92.22	85.57	carbendazim
Handwara	6	5	a	a	50.71	43.47	mancozeb
Nawpora	6	5	a	a	70.50	78.55	mancozeb carbendazim
Chogal	5	3	a	a	58.95	34.77	mancozeb carbendazim
Achabal	5	5	a	e	63.33	75.05	mancozeb carbendazim
Zakura	4	3	a	a	73.35	63.15	captafol
Narbal	4	4	a	a	76.78	76.54	mancozeb
Arihal	3	4	e	a	41.29	39.52	mancozeb
Nihalpora	3	2	a	e	36.36	53.26	mancozeb carbendazim

* e = single fungicide used exclusively
a = fungicides alternated

TABLE 2
Test fungicides permitting mycelial growth of different isolates of Venturia inaequalis on malt extract agar amended medium after 3 weeks at 18 ± 2 C.

Isolate from orchard	Resistance factor	
	Mancozeb	Carbendazim
Seer Jagir	2.0	3.0
Chitragam	2.5	1.0
Mattan Karewa	1.0	1.0
Thagiwara	1.5	1.0
Kriri	1.0	14.0
Imamsaheb	1.0	9.0
Handwara	2.0	1.0
Nawpora	2.5	1.0
Chogal	1.0	1.0
Achabal	1.0	1.0
Zakura	1.0	1.0
Narbal	1.0	1.0
Arihal	1.5	1.0
Nihalpora	1.0	1.0

Stability of resistance against carbendazim in Kriri, Imamsaheb and Seer Jagir isolates was found stable while these were unstable against mancozeb. All these resistant isolates proved pathogenic but lesions produced differed in nature and

virulence.

DISCUSSION

Orchards where application of unrelated fungicides in alternation in a six spray schedule was made had adequate disease control in comparison to those which received either exclusively mancozeb or carbendazim. The observations suggest that prolonged use of any one fungicide is not good.

Stability of resistance in V.inaequalis against carbendazim as observed in the present study has also been reported with other specifically acting fungicides (Wicks, 1976); while the unstability of resistance against mancozeb has also been reported with other unspecifically acting fungicides (Golyshin, 1964; Shibkora, 1966).

Pathogenic capability of the resistant isolates in present study suggests that if prolonged usage of any one fungicide is allowed, the selection pressure of such strains will lead to the failure of the control strategy as has been encountered in other countries (Szkolnik and Gilpatrick, 1969; Wicks, 1974).

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IMMUNOASSAY OF CARBOXYLESTERASE ACTIVITY FOR IDENTIFYING INSECTICIDE RESISTANT MYZUS PERSICAE

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ABSTRACT

An antiserum to carboxylesterase E4, the enzyme causing resistance of Myzus persicae to a wide range of insecticides, was used to measure the amount of this enzyme in individual aphids as a means of diagnosing resistance. The technique is simpler than methods involving electrophoretic characterisation or total esterase assay, and readily distinguishes susceptible from even slightly resistant (R_1) aphids.

INTRODUCTION

Peach-potato aphids (Myzus persicae) in many parts of the world have developed resistance to organophosphates, carbamates and pyrethroids (Georghiou 1981), usually, when so characterised, with cross-resistance between the different insecticide classes. Resistance in a given population is generally greatest to organophosphates and pyrethroids, and somewhat lower to carbamates (e.g. Sawicki & Rice 1978). Many instances of resistant M. persicae have been identified by bioassaying samples of populations collected from the field, which gives the average response of the, probably heterogeneous, population. However, by establishing and characterising clonal aphid populations, three major variants (S, R_1 and R_2) have been found to predominate on field crops in the U.K. (Sawicki et al. 1978), and a further two very resistant types are common in glasshouses (Devonshire 1977). Natural populations usually comprise a mixture of variants, with susceptible and slightly resistant (R_1) aphids most abundant in the field (Sawicki et al 1978) but with localised occurrence of the more resistant variant (R_2) especially in northern England (Sykes 1977) and Scotland (Devonshire et al. 1977).

The bioassay techniques for detecting resistance in aphids have been complemented in M. persicae by biochemical characterisation of the insects. The enzyme, carboxylesterase E4, responsible for resistance to a wide range of insecticides (Devonshire 1977, Devonshire & Moores 1982) can be assayed in a single aphid to give an indirect estimate of its resistance. By examining many individuals in this way, the proportion of different variants in a population can be measured (Baker 1977, 1978, Sawicki et al. 1978), and clonal cultures of the different variants established readily. Two techniques are commonly used to measure the E4 content of an aphid. Total esterase assay of a whole homogenate gives a quantitative measure of activity (Devonshire 1975) and is preferable when examining very resistant aphids since E4 contributes virtually all the activity. However, for slightly resistant M. persicae, other esterases common to all variants make a large contribution and can obscure the smaller difference in amount of E4 between these and susceptible aphids. In this case, electrophoretic analysis is preferable since it allows isolated E4 to be estimated, albeit subjectively, from the intensity of the stained band on the gel (Devonshire 1975, Baker 1977). Although the activity of E4 has been quantified on electrophoresis gels by spectrophotometric scanning (Blackman et al. 1977), this is not practicable on a large scale.

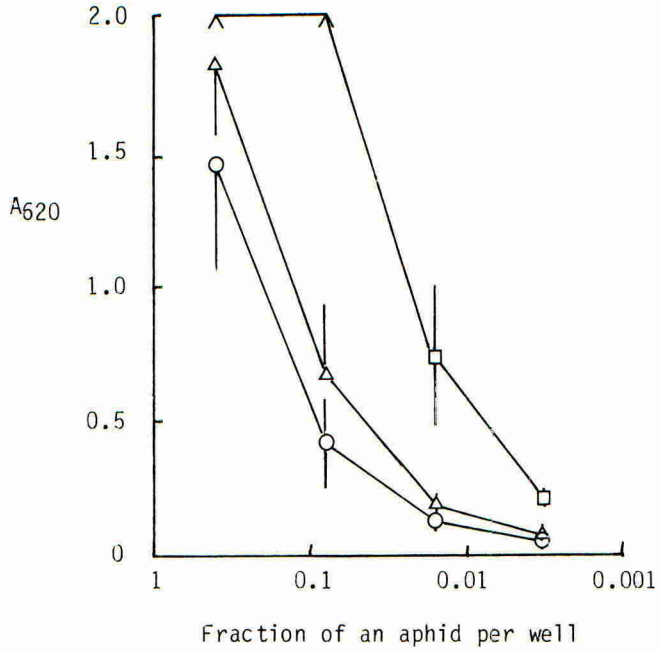

Fig. 1. Total esterase activity with naphth-1-yl acetate as substrate. Each point is the mean (± S.D. bars) of determinations on 3 different aphids from each clone: ○, S; △, R_1; □, R_2.

We report here a further technique that relies on trapping the E4 immunologically before its esterase activity is measured, so avoiding the "background" contribution of the other esterases.

MATERIALS AND METHODS

Aphids

The origins and rearing on excised potato leaves of the susceptible (S; clone US1L), slightly resistant (R_1; clone 405D) and very resistant (R_2; clone T1V) reference clones of *M. persicae* have been described previously (Sawicki *et al.* 1980).

Antiserum

E4 was purified from an homogenate of very resistant aphids (clone 740, equivalent to G6 in Sawicki *et al.* 1980) and shown to comprise only one detectable protein on SDS electrophoresis (Devonshire & Moores 1982). A rabbit was injected intramuscularly with *c.* 2 mg E4 emulsified in Freund's complete adjuvant and 4 months later with a further 2 mg in Freund's incomplete adjuvant. Blood samples were collected at 2-week intervals, and serum separated by centrifugation and stored at -20°C. Immunoglobulin G (IgG) was purified from the serum by affinity chromatography on Protein A-Sepharose CL-4B (see "Affinity Chromatography: Principles and Methods", Pharmacia Fine Chemicals) and stored at 4°C in phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl in 10 mM phosphate buffer, pH 7.4) at a protein concentration of 1.0 mg ml^{-1} and containing 0.02% sodium azice as preservative.

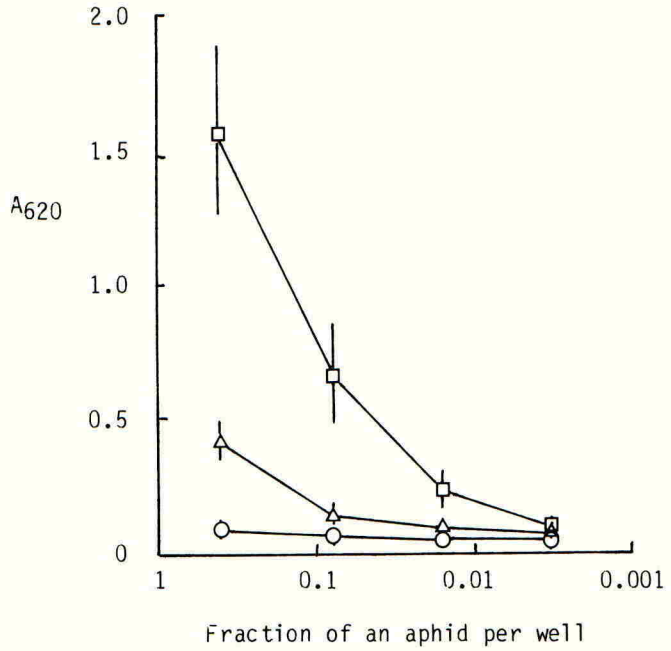

Fig. 2. IgG-trapped E4 esterase activity (same homogenates as in Fig. 1.) with naphth-1-yl acetate as substrate. See Fig. 1 for details.

Immunoassay

Principle

The method relies on adsorbing the IgG from the E4 antiserum to the wells of a polystyrene microtitration plate, using this to selectively bind E4 from a crude aphid homogenate and then measuring the bound esterase activity.

Microtitration plates

NUNC-Immunoplate II (96-well, flat bottom) microtitration plates were used after preliminary experiments with a number of different plates had shown the former to have the best protein-binding capacity and good reproducibility between both plates and wells.

Procedure

The plates were first 'coated' by incubation overnight at 4°C with 200 µl /well of IgG (diluted to 2 µg protein ml^{-1} coating buffer) (0.2 M sodium carbonate, pH 9.6), then thoroughly washed with 0.05% Tween 20 in PBS, and 200 µl of this same PBS/Tween added to every well.

Each aphid was homogenised with a glass rod in 100 µl PBS/Tween in a polyethylene microcentrifuge tube. Samples (50 µl) were added to the outer wells of the plate (already containing 200 µl PBS/Tween), mixed thoroughly and 50 µl removed and added to the next well in. By repeating these dilutions, a series of 4 wells was prepared for each aphid, containing c. 0.4, 0.08, 0.016 and 0.003 aphid. All dilutions and mixing were done simply and rapidly with a multichannel Finnpipette.

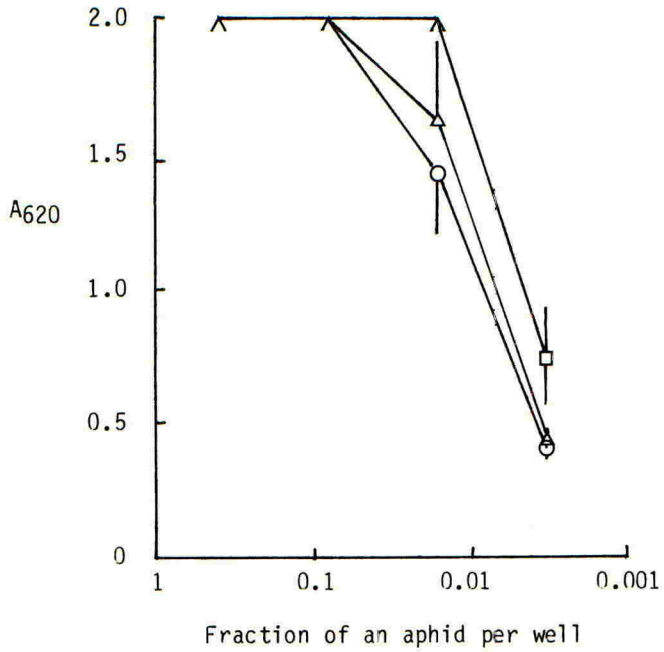

Fig. 3. Total esterase activity with naphth-1-yl butyrate as substrate. See Fig. 1 for details.

After 3 h incubation at 30°C, the plates were again washed with PBS/Tween and the esterase activity of the IgG-bound E4 assayed with either naphth-1-yl acetate or butyrate as substrate. The assay was essentially as described by Devonshire (1975), but using 200 µl substrate per well followed 30 min later by 50 µl diazo-blue-lauryl sulphate (DBLS). The absorbance in each well was both assessed by eye and measured at 620 nm in a Titertek Multiskan MC spectrophotometer 15 min later.

Incubations without IgG were done similarly in untreated microtitration plates to measure total esterase, as opposed to E4, activity; 50 µl aphid homogenate (diluted as appropriate) was added directly to 200 µl substrate, followed after 30 min incubation by 50 µl DBLS.

RESULTS AND DISCUSSION

The Figures show the mean absorbances obtained with 8 aphids using the two substrates, with and without IgG, as a function of homogenate dilution for the three clones, S, R_1 and R_2. With naphth-1-yl acetate, R_2 aphids are readily distinguished on the basis of total esterase activity, but there is considerable overlap between the data for S and R_1 aphids (Fig.1). These conditions and result are analogous to those in the 'tile test', useful as a very simple preliminary screen for very resistant aphids (Sawicki et al. 1978).

When the E4 from these same homogenates was selectively retained by binding to the IgG and then assayed with the same substrate (Fig. 2), the differences between clones became more clearcut so that even R_1 aphids were distinguished from S, as well as from R_2 insects.

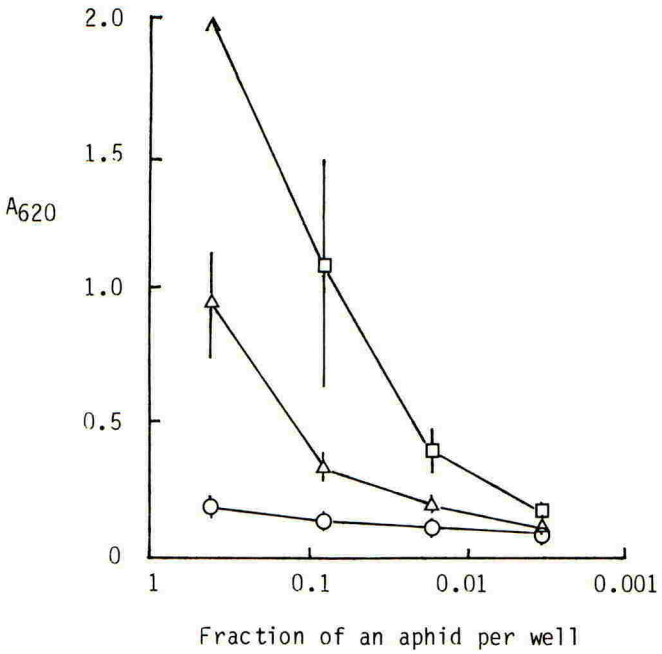

Fig. 4. IgG-trapped E4 esterase activity (same homogenates as in Fig. 3) with naphth-1-yl butyrate. See Fig. 1 for details.

Although purified E4 hydrolyses naphth-1-yl butyrate c. 8-fold faster than the acetate, other esterases, notably E1 and E2 (Devonshire 1977) are very much more active with the butyrate so they contribute proportionately more to the total esterase activity with this substrate in crude homogenates. This greater 'background' activity obscures the differences in amount of E4 between clones (Fig. 3). However, when used to assay IgG-trapped E4, naphth-1-yl butyrate gives good sensitivity and even clearer distinction between clones, especially S and R_1 (Fig. 4).

The data in Figs. 1-4 are based on assays of 4 dilutions of each aphid homogenate, so that 24 individuals are characterised in each microtitration plate. However, provided aphids from the standard clones are run in each experiment and the conditions are accurately controlled, the three variants should be distinguished by a single assay with naphth-1-yl butyrate, and homogenate equivalent to 0.2 aphid. When a known mixture of 96 of the three variants was so characterised, all 29 susceptible aphids were correctly identified and of the 35 R_1 individuals only 3 were wrongly classified as susceptible, and 6 as R_2. This small degree of uncertainty in identifying individual aphids is to be expected in view of the magnitude of the standard deviations. Although all experiments were assessed spectrophotometrically, evaluation by eye gave equally clear results.

This simple immunoassay thus readily distinguishes between the three M. persicae variants common in field crops in the U.K., even when the result is assessed by eye rather than spectrophotometrically. Although it has the disadvantage, compared to electrophoresis, of not revealing

qualitative variations in other esterases (not involved in resistance but useful as population 'markers'), it should prove a useful tool for studying resistance in field populations of this aphid since as many as 500-1000 aphids/day can be characterised compared to 100-150 with the other techniques of total esterase assay and electrophoresis.

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ESTERASE VARIATION IN THE BROWN PLANTHOPPER NILAPARVATA LUGENS AND ITS INVOLVEMENT IN RESISTANCE TO ORGANOPHOSPHORUS INSECTICIDES

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ABSTRACT

The susceptibility of eight populations of the brown planthopper Nilaparvata lugens was compared in the laboratory using five organophosphorus insecticides (malathion, diazinon, acephate, monocrotophos and carbophenothion). The Queensland strain was the most susceptible to the selected insecticides while the Japanese strain showed the most resistance, notably to malathion with a resistance factor of 409. The Philippine biotypes and carbofuran resistant strain showed resistance intermediate to that of the Japanese and susceptible strains. Total esterase activity of the hopper populations, measured using a colorimetric method, was highest in the most organophosphate resistant populations. Further investigation by polyacrylamide gel electrophoresis separated the esterases into eleven bands. Results from using selected inhibitors characterised the esterase bands as two arylesterases, one acetylcholinesterase and eight carboxylesterases. All resistant hoppers showed increased activity in one esterase, E10. The involvement of this esterase, a carboxylesterase, in resistance is discussed.

INTRODUCTION

Resistance to organophosphorus (OP) insecticides, notably malathion, has been linked to variations in esterase activity. The present study was conducted to determine if a correlation between insecticide resistance and esterase activity existed in the brown planthopper Nilaparvata lugens (BPH). Further characterisation of the esterases, by electrophoresis and the use of selected inhibitors, was undertaken to gain information on the types of esterase present in the BPH and to determine if a specific esterase was involved in resistance.

MATERIALS AND METHODS

Insect material

The origins of the BPH populations and rice cultivars used for culturing are given in Table 1. The biotypes from the International Rice Research Institute (IRRI), Philippines, are differentiated by their ability to colonise varieties of rice having different genes for conferring resistance to hopper attack. Therefore, rearing of the biotypes on suitable, susceptible rice varieties is essential for maintaining the correct biotype populations.

Bioassay

The large numbers of insects required during the bioassay necessitated the use of mixed populations of adult male and female hoppers along with a small percentage of 4th and 5th instar nymphs. The insecticides