CHARACTERISTICS OF *PSEUDOCERCOSPORELLA HERPOTRICHOIDES* ISOLATES RESISTANT TO PROCHLORAZ

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ABSTRACT

Prochloraz-resistant isolates of *P. herpotrichoides* were isolated from wheat stems in fields in different regions of France. Some of them of fast and slow growing type were used to inoculate plants in greenhouse experiments. Pathogenicity and competitive ability of sensitive and resistant strains are similar. The resistant strains seem to be as competitive or less competitive than the sensitive ones.

INTRODUCTION

Prochloraz and flusilazole are the most commonly used fungicides for controlling eyespot caused by *Pseudocercosporella herpotrichoides* in France. Prochloraz-resistant strains were isolated for the first time in 1990 on winter wheat (Leroux & Marchegay, 1991). Populations of *P. herpotrichoides* were monitored for sensitivity to prochloraz in 1990, 1991, 1992 and 1993. Few isolates were resistant to prochloraz in both the fast and the slow growing type (Cavelier *et al.*, 1992). In 1993, the percentage of isolates growing on 0.5 mg /l, 1 mg/l and 2 mg/l was 60, 27 and 9 % respectively (56 plots tested).

The knowledge of the fitness of the resistant strains may give some information about the use of prochloraz.

This paper presents the results of glasshouse experiments which compare two fitness parameters of resistant and sensitive isolates: pathogenicity and competition in the absence of fungicide.

MATERIALS AND METHODS

The isolates selected for this study were isolated from winter wheat in 1991 and 1992 in different regions of France. To test the sensitivity to prochloraz, each isolate was transferred to Petri dishes amended with 2 mg/l of the formulated compound (Cavelier *et al.* 1992). Experiments were carried out in a glasshouse heated only to give frost protection. Pots were arranged in a randomised design, with five blocks, and five plants per pot. Plants, cv. "Camp-Remy", were inoculated at the three leaf growth stage. The mean frequency of plants with symptoms was calculated at GS 60 (Zadoks *et al.* 1974). The severity of eyespot was assessed by the method of Cavelier & Le Page (1985). Plants were scored on a 0-2 scale: 0- healthy plants, 1- sheaths with lesions, 2- stems with lesions. Mean scores were calculated. On the stem, when eyespot lesions were present, the mean infected proportion of a cross-section of the stem was calculated.

Pathogenicity

Inoculation was carried out using a suspension of finely chopped mycelium. Four isolates were of the fast growing type, two were sensitive to prochloraz and two were resistant. Six isolates were of the slow growing type, three were sensitive to prochloraz, two were resistant. Isolates are designated resistant if they grow on 2 mg/l of prochloraz.

Competition

Plants were inoculated by spore suspension (100000/ml). Five mixtures of one resistant and one sensitive strain were made in different proportions: sensitive/resistant 100/0, 90/10, 50/50, 10/90, 0/100.

1: fast growing sensitive/ fast growing resistant
2: slow growing sensitive/ slow growing resistant
3: slow growing sensitive/ slow growing resistant
4: slow growing sensitive/ slow growing resistant
5: fast growing sensitive/ slow growing resistant

The strain mixtures were made with isolates of the same fields.

Isolations were made from stems with lesions to identify the strain originally involved.

RESULTS

Pathogenicity

As in previous work (Cavelier *et al.*, 1992), there was no difference between the pathogenicity of resistant and sensitive isolates (Table 1).

TABLE 1. Pathogenicity of prochloraz-sensitive or resistant isolates of *P.herpotrichoides*. (mean of 4 experiments)

Isolate type	mean frequency plant lesions	mean disease score 0-2	mean proportion stem cross-section infected
fast growing sensitive	82	1.6	58.9
fast growing resistant	78	1.6	57.6
slow growing sensitive	61	1.0	21.6
slow growing resistant	59	1.1	27.9

Competition

The pathogenicity of the mixture is higher than that of the single strains for the mixtures 2 and 5. (Table 2)

TABLE 2. Pathogenicity of mixtures of prochloraz-sensitive and resistant strains of *P. herpotrichoides* in known proportion. Mean disease score 0-2

	strains	proportion of sensitive/resistant strains 100/0	90.10	50/50	10/90	0/100
1	fast growing	1.4	1.5	1.2	1.4	0.6
2	slow growing	0.6	1.0	1.4	0.9	0.8
3	slow growing	0.8	1.2	1.2	0.7	1.2
4	slow growing	0.8	1.1	0.1	0.5	0.7
5	fast growing/ slow growing	0.6	1.2	1.6	0.7	0.8

The frequency of strains re-isolated from plants inoculated with a fast growing strain in the mixture was about the same for both strains. But, with a mixture of slow growing strains, the strains re-isolated were mainly sensitive ones.(Table 3)

TABLE 3. Mean frequency of prochloraz-resistant strains(%) re-isolated from plants inoculated with mixtures of prochloraz-sensitive and resistant strains of *P. herpotrichoides*, in known proportions.

Inoculated strains	Proportions of resistant strains % 10	50	90
fast growing	26	65	-
slow growing	0	0	10
fast growing/ slow growing	0	11	89

CONCLUSIONS

The similar pathogenicities of prochloraz-sensitive and resistant strains suggest that this parameter would have no influence in the evolution of populations of *P. herpotrichoides*. On the other hand, it was surprising that some mixtures of strains appeared more pathogenic than the single strains. These results differ from preceding observations, were similar mixtures of fast growing type/slow growing type were compared (Cavelier *et al.*, 1987).

The re- isolations from plants suggest that the resistant strains are equally competitive (fast-growing strains) or less competitive (slow-growing strains) than the sensitive ones. These results are in contrast to these seen with MBC resistant and sensitive strains, where the resistant strains were more competitive (Cavelier & Le Page, 1985), MBC resistance is now widely developed. This suggests that the evolution of prochloraz resistance could differ from that of MBC resistance.

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REDUCED SENSITIVITY TO THE EBI FUNGICIDE PROCHLORAZ IN THE CEREAL EYESPOT FUNGUS *PSEUDOCERCOSPORELLA HERPOTRICHOIDES*

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ABSTRACT

Isolates of *Pseudocercosporella herpotrichoides* reduced in sensitivity to prochloraz were derived from ultra-violet (UV) mutation and spontaneous selection on fungicide-amended media. Frequencies of resistance were found to be similar to those obtained for other ergosterol biosynthesis inhibiting (EBI) compounds. Although many of these putative mutants reverted rapidly to wild-type sensitivity, those generated from successive rounds of mutation or from spontaneous selection were found to be more stable. Mutants reduced in sensitivity to prochloraz were compared with wild-type isolates in terms of response to prochloraz *in vitro* and *in vivo*, growth rate, sporulation ability, pathogenicity and competitive ability on a range of cereal hosts. The observations made from this study support a multi-factorial basis for prochloraz resistance, suggesting directional selection for field resistance.

INTRODUCTION

Eyespot, caused by the fungus *Pseudocercosporella herpotrichoides* (Fron.) Deighton, is an important disease of winter cereals with annual losses averaging £20 - 30 million in the UK alone. In the 1970's disease control was provided by application of the methyl-benzimidazol-2-yl carbamate (MBC) fungicides. However, wide-spread resistance had developed to this group by the early 1980's (King and Griffin, 1985) and de-methylation inhibitors such as prochloraz are currently recommended for eyespot control in the U.K.

Prior to 1990, there were no published cases of reduced control of *P. herpotrichoides* by prochloraz. Strains with reduced sensitivity had been recovered from field surveys (Gallimore *et al.*, 1987) but tolerance levels were below the recommended application rate. In 1990, strains showing reduced-sensitivity to prochloraz at levels of up to 10 mg/l agar were isolated from northern France (Leroux and Marchegay, 1991). This discovery emphasised the need for further research into the development of resistance in the field.

In the current study, strains of *P. herpotrichoides* with reduced sensitivity to prochloraz were mass-selected on fungicide-amended media or induced by UV-irradiation. The stability of resulting putative mutants was assessed through serial transfer on amended and unamended media, sporulation steps and passage through plants. Stable isolates were

further characterised in terms of fitness by measuring growth rate in culture, pathogenicity and ability to compete with the sensitive wild-type after co-inoculation on cereal hosts.

MATERIALS AND METHODS

Production of mutant strains

Three field isolates of *P. herpotrichoides* were used in this study: R-types 22-12 and 22-1011 and a W-type 22-20. Isolates reduced in sensitivity to prochloraz were induced by UV irradiation of conidia or spontaneous selection on prochloraz-amended media using the method described by Julian *et al.* (1994 a). Putative mutants were purified on malt-yeast-glucose (MYG) agar (5 g malt extract, 2.5 g yeast extract, 10g glucose, 20 g agar l^{-1} distilled water) amended with 5 μM prochloraz (1 μM = 0.377 mg/l) and then serially sub-cultured three times on amended and unamended media to confirm stability. A further two rounds of UV mutagenesis were carried out on selected mutants derived from 22-12, resulting in the production of resistant strains classified as intermediate or high level mutants.

Characterisation of mutants

In vitro plate assays were used to assess the dose response to prochloraz of the mutant strains. MYG plates amended with a range of concentrations (0.1 - 200 μM) of technical grade prochloraz were inoculated with 3 mm plugs cut from the growing edge of a colony. For each treatment 6 replicate colonies were measured after 14 days at 19°C, and the percent inhibition calculated from the unamended control. This data was used to determine the LC_{50} and MIC values for each isolate. The dose response was re-tested after a 1 year period during which time isolates were sequentially sub-cultured on amended and un-amended medium. The response of strains was also tested following sporulation steps and passage through wheat cv. Avalon.

Pathogenicity was tested by inoculating 21 day old seedlings of Avalon with conidial suspensions of the test isolates (2 x 10^5 ml^{-1}) (Daniels *et al.*, 1991). For all treatments 45 plants in 9 replicate pots were assessed. Plants were maintained in a growth room (16 h daylength, 17 ($\pm$ 2)°C) for 6 - 7 weeks after which symptoms were visually scored (Scott, 1971). For fungicide treatments plants were sprayed with Sportak 40, at a rate equivalent to 200 g A.I./ha, 24 hours prior to inoculation. Re-isolations taken from infected stem-base material were tested to confirm level of sensitivity to prochloraz. For competition studies 10 day old seedlings of Avalon were co-inoculated as previously described with mixtures of the sensitive wild-type parent 22-12, and either the spontaneously selected strain 22-1103, or the UV-induced mutant 22-1128. Conidia were mixed in the following ratios: 100:0, 75:25, 50:50, 25:75, 0:100. Prochloraz was applied, at a rate equivalent to 400 g A.I./ha, 4 days after inoculation. Plants were visually assessed after 12 weeks, and 1 cm stem sections surface-sterilised prior to re-isolation to determine the proportion of sensitive and resistant fungal outgrowths.

RESULTS

Frequencies of spontaneous resistance to prochloraz were within the range of 10^{-6} to

10^{-7}. UV-irradiation increased this frequency to approximately 10^{-5} (2 - 20% survival). Although many putative mutants reverted rapidly to wild-type sensitivity, those produced after several rounds of mutagenesis proved to be relatively stable in culture (Figure 1). Sporulation steps or passage through cereal hosts did result in reduced levels of resistance in some UV-induced isolates, however, resistance levels of spontaneously selected mutants were less affected by these treatments (Julian *et al.*, 1994a).

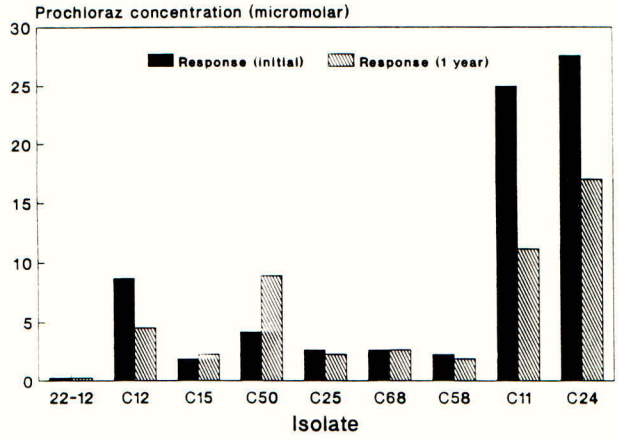

Fig. 1. The LC_{50} to prochloraz (μM) of UV-induced mutants after 1 year of continuous serial sub-culture, compared with the LC_{50} response immediately after isolation and purification.

Growth rates in culture and sporulation ability of mutant isolates were variable but there was no correlation between resistance level and growth rate (R-squared = 0.051), or conidia production (R-squared = 0.006). No reductions in pathogenicity were associated with spontaneous mutants. However, UV-induced mutants were more variable (Figure 2).

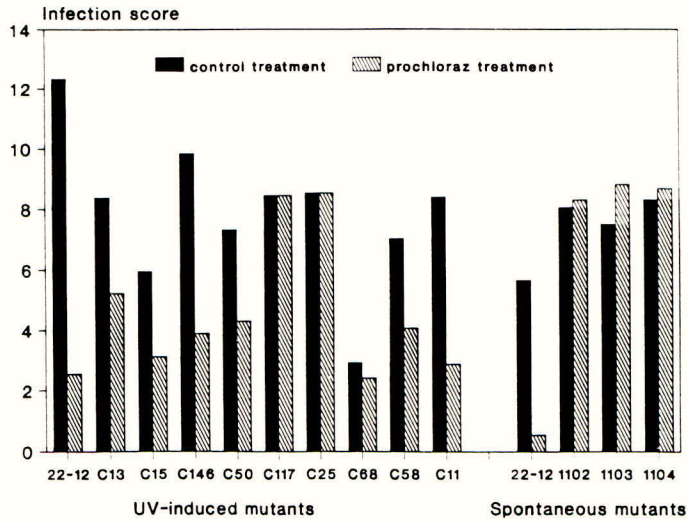

Fig. 2. Pathogenicity of UV-induced and spontaneous mutants from the R-type 22-12, on wheat cv. Avalon, in the presence and absence of a prochloraz treatment.

TABLE 1. The sensitivity of colonies re-isolated from Avalon stem bases inoculated with mixtures of the wild type 22-12, and the spontaneously selected strain 22-1103, or the UV-induced resistant strain 22-1128 to prochloraz.

Inoculum mix	Number of colonies re-isolated			
	Fungicide treated		Untreated	
	Sensitive	Resistant*	Sensitive	Resistant*
22-12:22-1103				
100:0	0	1	11	0
75:25	2	110	1	31
50:50	3	90	3	28
25:75	4	28	0	3
0:100	6	87	0	29
22-12:22-1128				
100:0	0	1	11	0
75:25	2	32	3	9
50:50	0	62	8	48
25:75	0	29	1	0
0:100	0	25	0	26

* Ability to grow on MYG amended with 5 μM prochloraz

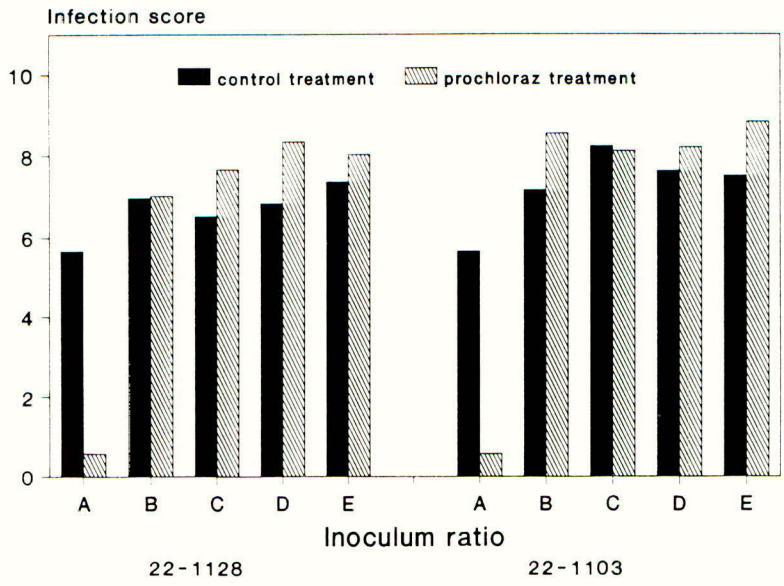

Fig. 3. The effect of co-inoculating the wild-type 22-12 with insensitive mutants 22-1128 (UV-induced) and 22-1103 (spontaneous) on disease levels on wheat cv. Avalon. Inoculation ratios (22-12:mutant): A = 100:0, B= 75:25, C = 50:50, D = 25:75, E = 0:100.

Re-isolations from stems infected during the competition experiments comprised a mixture of sensitive and resistant material (Table 1). There were no significant differences between infection scores on co-inoculated plants but plants inoculated solely with the sensitive wild-type 22-12 had significantly reduced infection scores ($P < 0.001$) particularly where a prochloraz treatment had been applied. Disease scores were often higher on prochloraz treated plants inoculated with the insensitive isolates (Figure 3).

DISCUSSION

Assessment of the characteristics of isolates of *P. herpotrichoides* resistant to prochloraz is vital when evaluating the likelihood of establishment of such strains in field populations. In order for resistance to become established, resistant strains must arise and be able to persist in the field. Factors such as rate of mutation to the resistant phenotype, selection pressure exerted by the fungicide, stability and competitive ability of the mutants and mode of dissemination will all contribute to this. The recent occurrence of prochloraz resistant strains in France suggests that these criteria can be satisfied under field conditions (Leroux and Marchegay, 1991). Although it is well-established that conidia are distributed by rain-splash (Fitt and Bainbridge, 1983), the role played by ascospores is still unclear. However, apothecia were found to occur at numerous set-aside sites throughout England during 1993 (Dyer, pers. comm.).

In this study, frequency of resistance to prochloraz after UV mutation was found to be similar to that identified with other EBI compounds. For example, induction of triadimenol resistance in W-type isolates of *P. herpotrichoides* gave frequencies of 5×10^{-5} for a 90% kill (Leroux *et al.*, 1988). Levels of resistance observed in the prochloraz-resistant mutants were similar to those derived from studies with imazilil, an imidazole closely related to prochloraz, with *Aspergillus nidulans* (van Tuyl, 1977). Generation of increasing numbers of mutants with successive rounds of UV mutation, along with higher resistance levels of third round mutants, may suggest a multigenic trait involving progressive alteration of more than one gene.

Most UV mutants were found to be unstable, rapidly reverting to wild-type sensitivity after mutation. The resistance initially expressed may have been of a transient nature, possibly from physiological adaptation to the fungicide. Third-round UV mutants and low level spontaneous mutants were found to be stable, even after one year in culture, possibly indicating a different resistance mechanism. Previous studies of cultural instability in the pathogen, showed that morphologically altered sectors had increased sensitivity to prochloraz compared to the parent colony. This may have important implications for resistance testing and may be indicative of a degree of instability of this type of resistance in culture (Julian *et al.*, (1994b).

Differences in pathogenicity levels on cereal hosts between classes of mutant may also indicate different resistance mechanisms, with the possibility that the mutation procedure had pleiotropic effects causing a reduction in infective ability. Additional studies found that field isolates showing reduced sensitivity to prochloraz gave higher infection scores in the presence of the fungicide than on untreated control plants (Hardy, unpublished). Co-inoculation with sensitive and resistant isolates indicated that resistant isolates were able to compete effectively with the susceptible parent.

Evidence from this study supports the hypothesis that development of resistance to prochloraz will be directional in nature, with the possibility that small incremental changes in resistance level, reflecting a multi-factorial basis for the trait, will occur. Establishment of the genetic nature of prochloraz resistance could lead to the identification of molecular markers. These could provide improved diagnostic procedures for field surveys and enable the development and spread of resistance to this fungicide to be more readily and accurately followed.

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EVALUATION OF HYPHAL ELONGATION AS A BASIS FOR MONITORING THE SENSITIVITY OF *VENTURIA INAEQUALIS* AND *MYCOSPHAERELLA FIJIENSIS* TO FLUSILAZOLE

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ABSTRACT

An *in vitro* procedure, based on elongation of primary hyphae, was developed to monitor the sensitivity to flusilazole of *Venturia inaequalis* and *Mycosphaerella fijiensis*. The lengths of primary hyphae from 50 pathogen spores, transferred from infected plant tissue, were compared after 2±0.5 days of growth on flusilazole-amended and unamended agar media. Evaluations were made when hyphae on unamended media had elongated significantly without obscuring the original spores and were not overrun by contamination. For each flusilazole concentration, isolates were considered inhibited if the lengths of their primary hyphae were ≤50% of the hyphal lengths on unamended media. Minimum inhibitory concentrations determined by hyphal elongation were equivalent to those obtained by colony radial growth. These results demonstrate that the hyphal elongation assay is a simple, rapid, and accurate method for monitoring the sensitivity of these pathogens to flusilazole.

INTRODUCTION

Flusilazole is a demethylation-inhibiting (DMI) fungicide developed by DuPont, which is effective against a broad spectrum of plant diseases (Fort & Moberg, 1984). Essential to our stewardship of flusilazole is the development and use of techniques to accurately assess the sensitivities of pathogens to this fungicide. This paper describes a novel *in vitro* procedure, based on the determination of primary hyphal elongation, to monitor the sensitivity to flusilazole of *Venturia inaequalis*, the cause of apple scab, and *Mycosphaerella fijiensis*, incitant of black sigatoka of bananas.

MATERIALS AND METHODS

Sample collection

Apple leaves exhibiting young, well-defined sporulating lesions of *V. inaequalis* were collected in 1988 in a randomized pattern from 3 U.S. orchards with and without DMI fungicide treatments, refrigerated in paper bags, and processed within 5 days of collection. Banana leaves with *M. fijiensis*-infected necrotic tissue containing ripe perithecia were obtained in 1990 from 3 commercial plantations in Central America, 2 weeks following the last DMI application; as well as from 1 location untreated with DMI fungicides. Leaf tissue was refrigerated dry in plastic bags until testing within 2 weeks of collection.

Media preparation

Technical flusilazole dissolved in acetone or formulated product in water was added at the appropriate concentrations to cooled, molten media (45°C) after autoclaving, mixed thoroughly, and poured into petri plates. Solvent was added to unamended media.

Hyphal elongation assay

V. inaequalis spores were transferred from 1 lesion per leaf as 5 short streaks onto the surface of potato dextrose agar (PDA) that was unamended or amended with 0.005, 0.01, or 0.05 mg/l AI flusilazole in each petri plate. Petri plates were incubated for 2±0.5 days at 21°C with 12-h day.

Banana leaves were first incubated for 48 hours at 20-25°C in a plastic bag with a moist towel to allow ascospore maturation. Five leaf sections (1-2 cm^2) from different locations were stapled to each 9-cm filter paper disc, immersed in water for 5 minutes, and placed inside a petri plate cover over 2% water agar that was unamended or amended with 0.001, 0.01, 0.1, 1.0, or 2.5 mg/l AI flusilazole for 1 hour to allow discharge of ascospores. Petri plates were incubated for 2±0.5 days at 26°C with 12-h day.

The lengths of primary hyphae from 50 spore streaks (*V. inaequalis*) or spores (*M. fijiensis*) were determined on amended and unamended media at 100x magnification using either an ocular micrometer or as multiples of spore length (Smith, Johnson *et al.*, 1991; Smith, Trivellas *et al.*, 1991). Evaluations were made when the primary hyphae on unamended media had grown a significant amount without obscuring the original spores and before saprophytic contamination overran pathogen hyphae. Daily examination of petri plates was necessary since the optimal time for assessment varied slightly (±0.5 day) depending on the growth rates of the pathogens and saprophytes. For each flusilazole concentration, isolates were considered inhibited if the lengths of their primary hyphae were ≤50% of the hyphal lengths on unamended media. For *V. inaequalis*, the response of the majority of spores in a streak was used to describe the entire streak due to the uniformity in response. For *M. fijiensis*, the percentage of reduction in hyphal length on flusilazole-amended media compared to the untreated controls was determined. Minimum inhibitory concentrations (MIC) for both pathogens were established.

Colony radial growth assay

Responses in the hyphal elongation assay were determined for 40 spore streaks or individual spores of wild-type *V. inaequalis* or *M. fijiensis* isolates, respectively. These propagules were then transferred to PDA (*V. inaequalis*) or Mycophyll® Agar (*M. fijiensis*) media that were unamended or amended with the flusilazole concentrations corresponding to those in the hyphal elongation assay. Colony diameters were determined after 2 weeks and compared to hyphal elongation responses.

RESULTS

Hyphal elongation assay

Spores of *V. inaequalis* and *M. fijiensis* isolates unexposed or exposed to DMI fungicides in the field initially germinated and transverse cell walls formed in germ tubes on media that were unamended or amended with flusilazole at all concentrations evaluated. However, after approximately 2 days, elongation of primary hyphae ceased on higher concentrations of flusilazole, whereas hyphal growth continued on unamended media and lower flusilazole concentrations (Table 1a,b).

At the time of evaluation, hyphal elongation in all spore streaks of the *V. inaequalis* isolates unexposed to DMI fungicides in the field was significantly inhibited at 0.05 mg/l AI flusilazole compared to unamended controls, whereas growth inhibition occurred in a decreasing number of spore streaks at 0.01 and 0.005 mg/l (Table 1a). An MIC between 0.01 and 0.05 mg/l AI flusilazole was determined. Isolates that were unexposed or exposed to DMI fungicides in the field provided similar dosage responses and MIC values.

Hyphal elongation in the *M. fijiensis* isolate unexposed to DMI fungicides in the field was completely inhibited at ≥0.1 mg/l AI flusilazole concentrations but not inhibited at ≤0.01 mg/l flusilazole (Table 1b). An MIC between 0.01 and 0.1 mg/l AI flusilazole was established. Isolates with and without exposure to DMI fungicides in the field had similar dosage responses and MIC values.

TABLE 1. Primary hyphal elongation of isolates of *Venturia inaequalis* (a) and *Mycosphaerella fijiensis* (b) on agar media amended with flusilazole compared to unamended controls.

a)

Isolate origin	Orchard treated with DMI Fungicides	% spore streaks with primary hyphal lengths ≤50% of unamended controls		
		0.005*	0.01	0.05
Penna.	No	5	58	100
New York	No	0	67	100
Delaware	Yes	3	90	100

*mg/l AI flusilazole

b)

Isolate origin	Plantation Treated with DMI fungicides	% spores with primary hyphal lengths ≤50% of unamended controls				
		0.001*	0.01	0.1	1.0	2.5
S. Catalina, HN	No	0	0	100	100	100
S. Alberto, CR	Yes	0	0	100	100	100
S. Barbara, HN	Yes	0	0	100	100	100
Tacamiche, HN	Yes	0	0	100	100	100

*mg/l AI flusilazole

Colony radial growth assay

For *V. inaequalis* and *M. fijiensis*, the respective levels of hyphal elongation and colony radial growth inhibition relative to untreated controls were comparable at all concentrations of flusilazole tested (Table 2a,b). In both the hyphal elongation and radial growth assays, the *V. inaequalis* isolate was completely inhibited at 0.05 mg/l AI flusilazole and partially inhibited at 0.005 and 0.01 mg/l (Table 2a). An MIC between 0.01 and 0.05 mg/l AI flusilazole was established for both assays.

Primary hyphal elongation and colony growth of the *M. fijiensis* isolate on 0.1 and 1.0 mg/l AI flusilazole were completely inhibited, whereas spores on 0.001 and 0.01 mg/l AI flusilazole developed as those on unamended media (Table 2b). An MIC between 0.01 and 0.1 mg/l AI flusilazole was determined for both assays.

TABLE 2. Comparison between primary hyphal elongation and colony radial growth of isolates of *Venturia inaequalis* (a) and *Mycosphaerella fijiensis* (b) on flusilazole-amended media, relative to unamended controls.

a)

Flusilazole Concentration (mg/l)	% spore streaks with primary hyphal lengths ≤50% of unamended controls	% inhibition of colony radial growth compared to unamended controls
0.005	7	21
0.01	71	79
0.05	100	100

b)

Flusilazole Concentration (mg/l)	% spores with primary hyphal lengths ≤50% of unamended controls	% inhibition of colony radial growth compared to unamended controls
0.001	0	0
0.01	0	0
0.1	100	99
1.0	100	100

CONCLUSIONS

The hyphal elongation assay is based on the effects on primary hyphal growth due to inhibition of sterol biosynthesis by flusilazole. Initial germination and germ tube growth of spores are not sensitive to sterol biosynthesis inhibition and their responses cannot be used to monitor sensitivity to flusilazole.

Use of the hyphal elongation assay to monitor the sensitivity to flusilazole of a limited number of isolates of *V. inaequalis* and *M. fijiensis* from locations in the U.S. and Central America, respectively, showed no difference in responses between isolates unexposed and exposed to DMI fungicides in the field.

Based on the results presented in this paper, the hyphal elongation assay is a simple, rapid, and accurate method for monitoring sensitivity of fungal pathogens to flusilazole. Comparisons between hyphal elongation and radial growth assays show excellent agreement. The hyphal elongation assay may be generally applicable to monitoring the sensitivity of other pathogens to various DMI fungicides.

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MONITORING STUDY OF THE RESISTANCE OF *Erysiphe graminis* f. Sp. *tritici* TO EBI FUNGICIDES IN HUNGARY

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ABSTRACT

A programme was initiated in 1993 to monitor the sensitivity of *Erysiphe graminis* f. sp. *tritici* to ergosterol biosynthesis inhibiting fungicides (EBIs) in Hungary. In 1993, 41 isolates from 11 countries were tested for sensitivity to triadimefon and tridemorph, using a test tube assay. The tridemorph sensitivities of all the isolates were similar to that of a standard isolate. Triadimefon sensitivities were more widely distributed, with LC50 values ranging from <2mg/l to 10mg/l. The LC50 values of only two isolates exceeded 10mg/l. These results will be used for comparison in future years, as part of a long term resistance monitoring programme.

INTRODUCTION

In Hungary, cereals are grown on 26 to 30 % of the arable acreage, of which the share of wheat is 85 to 90%. Powdery mildew is one of the limiting factors affecting profitability. The importance of *Erysiphe graminis* on wheat has increased in Hungary in recent years. As described by Szunics (1988), it causes an average of 5-8% yield loss, though in extreme cases this may be 20-30%.

Based on the severity of disease, 1 or 2 chemical treatments may be required. In addition to contact fungicides, systemic fungicides (such as benzimidazoles and EBIs) are available. The EBIs are very effective, broad spectrum fungicides, but their efficacy can be influenced by decreases in sensitivity of the pathogen (Brent & Hollomon, 1988; Schulz and Scheinpflug, 1986). Resistance of *E. graminis* to DMI and morpholine fungicides in Western Europe is reported in publications by researchers in the FRAC-SBI Committee (De Waard *et al.*, 1986; Highwood, 1990; Brent, 1992). Earlier studies in Western Hungary were made by Enisz (1988, 1990) on the resistance of *E. graminis* to DMIs, but no

nationwide monitoring of resistance has yet been carried out in Hungary. Thus, it was thought necessary to survey the status of DMI and morpholine sensitivity and to initiate a monitoring study on resistance, to be carried out on a regular basis. Studies started in 1991 with the assistance of a grant obtained for research and development.

MATERIALS AND METHODS

Sampling areas were chosen from wheat growing regions of Hungary, where conditions are especially conducive to mildew development. Because of the long drought, only 49 wheat samples were collected from 11 counties. The pathogen was successfully isolated from 41 samples.

Test plant

The mildew-sensitive wheat variety, GK.Öthalom, was used for isolating the pathogen and for further propagation and sensitivity studies.

Fungicides

Tests were carried out with Bayleton 25% WP (triadimefon) the product most widely used on wheat in Hungary since 1978, and with Calixin (tridemorph) registered on wheat since 1988, with the following application rates (AI (mg/l): 0.3; 1; 3; 10; 30; 100.

Sampling, isolation of the pathogen and further propagation

20-25 infected plants were sampled per field. Mildew samples were sub-cultured once on to healthy wheat plants at the 1-leaf growth stage, prior to sensitivity testing.

Fungicide sensitivity test

The "test tube method for assessment of propiconazole sensitivity in cereal powdery mildew isolates" by Sozzi *et al* (GIFAP, 1991) was selected from the methods recommended by FRAC for monitoring fungicide resistance. A culture maintained since 1986 on wheat cv. GK.Öthalom was used as the standard sensitive isolate.

RESULTS

Before analysing the results, the following should be noted:

Because of the long dry weather prevailing in Hungary in 1993, the level of *E. graminis* infection was "low" on 29%, "medium" on 58% and "high" on only 13% of the sampled area.

- *c.* 30% of the sampled area was treated with one application of fungicide to control powdery mildew.
- DMI fungicides were applied to 58% of the treated fields. Morpholines were not applied.
- 20% of the treated fields were cultivated as monoculture.
- The 41 samples studied were taken from 17 wheat varieties.

Table 1 shows the LC50 values (mg/l) and the resistance factors.

Table 1. Results of *Erysiphe graminis* f.sp. *tritici* resistance monitoring programme 1993.

ISOLATES		TRIADIMEFON		TRIDEMORPH	
No.	County of Origin	LC50	RF	LC50	RF
1.	Nógrád	0.2	0.1	0.6	0.5
2.		0.2	0.1	0.7	0.8
3.		0.6	0.4	0.7	0.8
4.		2.3	1.5	0.6	0.5
5.		1.7	1.1	1.2	1.0
6.		0.6	0.4	0.3	0.2
7.		0.7	0.5	0.9	0.7
8.		1.7	1.1	0.2	0.2
9.	Somogy	4.3	2.9	1.2	1.0
10.		0.5	0.3	0.8	0.7
11.		13.0	8.7	1.5	1.2
12.		8.0	5.3	1.5	1.2
13.		1.8	1.2	0.2	0.2
14.	Veszprém	2.6	1.7	0.3	0.2
15.		2.0	1.3	0.4	0.3
16.		3.3	2.2	0.5	0.4
17.		1.0	0.7	1.4	1.2
18.		8.0	5.3	1.6	1.3
19.		3.1	2.1	1.8	1.4

Table 1. Results of *Erysiphe graminis* f.sp. *tritici* resistance monitoring programme 1993 (continued).

ISOLATES		TRIADIMEFON		TRIDEMORPH	
No.	County of Origin	LC50	RF	LC50	RF
20.	Pest	1.5	1.0	1.5	1.2
21.		7.0	4.7	1.1	0.9
22		1.9	1.3	1.4	1.2
23		2.0	1.3	1.8	1.4
24		3.0	2.0	1.7	1.3
25	Bács-Kiskun	7.5	5.0	1.1	0.9
26		17.0	11.3	1.6	1.3
27		0.2	0.1	1.2	1.0
28		1.7	1.1	1.1	0.9
29		0.3	0.2	1.2	1.0
30.	Komárom-Esztergom	6.5	4.3	0.4	0.3
31.		1.5	1.0	0.6	0.5
32.		1.8	1.2	0.5	0.4
33.		0.4	0.3	0.3	0.2
34.		0.9	0.6	0.3	0.2
35		2.8	1.9	2.0	1.7
36	Szolnok Heves	1.6	1.1	1.3	1.1
37		0.8	0.5	0.3	0.2
38.		0.6	0.4	0.9	0.7
39.		0.6	0.4	0.2	0.2
40.	Győr	1.1	0.7	1.6	1.3
41.		0.5	0.3	0.5	0.4
	STANDARD	1.5		1.2	

Tridemorph sensitivity

The LC50 values of most isolates were similar to that of the standard isolate (1.2mg/l). No isolate had an LC50 value greater than 2mg/l. The resistance factor did not exceed 2, for any of the isolates.

Triadimefon sensitivity

With triadimefon as the active ingredient, the values were more widely distributed. However 66% of the isolates had LC50 values below 2mg/l. (The LC50 value of the standard isolate was 1.5mg/l). 17% and 12% of the isolates had LC50 values in the 2-4mg/l and 4-8mg/l categories, respectively. The LC50 values of only 2 isolates exceeded 10mg/l. The value of the R-factor of only one isolate was higher than 10.

DISCUSSION

The main objective of this study was to provide data at the start of a long term programme for monitoring fungicide resistance in *E. graminis* in Hungary. The results from 1993 are published here. When evaluating these results, the dry weather and consequent reduction in number of fungicide treatments should be taken into account. However, the results provide valuable information at the start of the resistance monitoring programme.

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