Session 1
DMI Fungicides

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RESISTANCE TO FUNGICIDES WHICH INHIBIT STEROL 14α-DEMETHYLATION, AN HISTORICAL PERSPECTIVE

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ABSTRACT

This paper gives an historical perspective on fungal resistance to fungicides which inhibit sterol 14α-demethylation (DMIs). Topics covered are resistance development in the field, the genetics of resistance and the biochemical mechanisms involved. At present, reduced sensitivity or resistance to DMIs has been reported for at least 13 plant pathogens. In most of these fungi, and in laboratory-generated mutants, resistance is polygenic. Cases of monogenic resistance have also been published. A variety of biochemical resistance mechanisms may operate. Resistance caused by decreased affinity of sterol 14α-demethylase to DMIs has not yet been established. Other mechanisms, such as increased efflux from fungal mycelium and changes in the sterol biosynthetic pathway have been reported, although the relevance of these mechanisms for resistance in plant pathogens remains to be elucidated.

INTRODUCTION

From 1969 onwards at least 36 sterol 14α-demethylation inhibitors (DMIs) were introduced as agricultural fungicides. Remarkable features of these type of compounds are their high protective and curative properties, low use rates, systemic and vapour phase activity, high chemical variability leading to diverse spectra of antifungal activity, and selective action between target and non-target organisms (De Waard, 1993). Nowadays, the use of fungicides in various crops is dominated by DMIs. Hence, the successful introduction of DMIs clearly represents a landmark in chemical disease control.

Development of resistance to fungicides with a specific mode of action is a major threat to effective chemical disease control. By 1969, serious resistance problems in many plant pathogens were already encountered with different site-specific fungicides. DMIs also have a site-specific mode of action. Therefore, it is understandable that these fungicides received timely attention with respect to the resistance risk. Fuchs and Drandarevski (1976) stated that development of resistance to DMIs under practical conditions would be rather unlikely. History has demonstrated that this statement was premature, since DMI-resistance has now been reported in various plant pathogens. However, resistance developed relatively slowly as compared with other classes of site-specific fungicides.

This paper gives an historical overview of resistance development to DMI fungicides in practice, its genetic basis, and the mechanisms of resistance involved.

RESISTANCE DEVELOPMENT

Assessment of resistance to DMIs is difficult since the level of resistance is often so low that its development could only be detected when proper background sensitivity studies were previously made. The presence of strains with decreased sensitivity does not necessarily imply

TABLE 1. Chronological list of reports on reduced sensitivity and/or field resistance to DMI fungicides in plant pathogens.

Pathogen	Crop	Authors
Erysiphe graminis f.sp. *hordei*	Barley	Fletcher and Wolfe, 1981
Sphaerotheca fuliginea	Cucumber	Schepers, 1983
Pyrenophora teres	Barley	Sheridan *et al.*, 1985
Venturia inaequalis	Apple	Stanis and Jones, 1985
Erysiphe graminis f.sp. *tritici*	Wheat	De Waard *et al.*, 1986
Rhynchosporium secalis	Barley	Hunter *et al.*, 1986
Penicillium digitatum	Citrus	Eckert, 1987
Uncinula necator	Grape	Steva *et al.*, 1990
Pseudocercosporella herpotrichoides	Wheat	Leroux and Marchegay, 1991
Botrytis cinerea	Vegetables	Elad, 1992
Mycosphaerella fijiensis	Banana	Anonymous, 1992
Puccinia horiana	Chrysanthemum	Cevat, 1992
Septoria tritici	Wheat	Hollomon (pers. comm., 1993)

loss of field control by a particular DMI (*e.g. Septoria tritici*). This depends on the level of resistance and the frequency of resistant strains. In this paper, the term "field resistance" is used for situations where these parameters coincide. The term "reduced sensitivity" is used for practical situations in which the presence of a pathogen population with decreased sensitivity to a DMI does not obviously result in decreased field performance.

Table 1 gives an overview of reports in which reduced sensitivity and/or resistance in field isolates of plant pathogens were described for the first time. Later reports on similar cases are not cited in this paper but often concur with the reported data or indicate that levels of resistance have increased over several years. The latter phenomenon has, for instance, been described for resistance to various DMIs in *Sphaerotheca fuliginea* in the Netherlands (Schepers, 1985), *Venturia inaequalis* in Canada (Hildebrand *et al.*, 1988), and *Erysiphe graminis* f.sp. *hordei* in the UK (Heaney, 1988). Despite this, the data mentioned in Table 1 have to be handled with care, since resistance may stabilize in different regions and under different conditions at different levels. For instance, despite the early report on resistance development, various DMIs still effectively control *S. fuliginea* in glasshouse cucumbers in the Netherlands, while failures of disease control in field-grown cucurbits are common in some Mediterranean countries (Huggenberger *et al.*, 1984). Barley powdery mildew in the UK became field resistant to some triazoles, while similar compounds are still effective against wheat powdery mildew in other European countries. Poor disease control or relatively short persistence of action by particular DMIs may also only become evident if disease conducive conditions coincide with the presence of pathogen populations with reduced sensitivity (De Waard *et al.*, 1986). Although cross-resistance usually extends to all DMIs, some DMIs, especially the recently introduced ones, may remain highly effective against cereal powdery mildews. This may be due to their relatively high activity, and to differences in levels of cross resistance to different DMIs (De Waard, 1992[a]).

Inclusion of some pathogens in Table 1 may be subject to dispute. This is the case for *Pseudocercosporella herpotrichoides* since Birchmore *et al.* reported in 1992 no major changes in sensitivity to prochloraz in populations of the pathogen in various European countries. Resistance

of *Pyrenophora teres* to triadimenol could be a case of natural insensitivity, since insensitive isolates in New Zealand are present in the natural population of the pathogen (Sheridan *et al.*, 1985). Reduced sensitivity of *Botrytis cinerea* to DMIs was reported in Israel in field tests to study the efficacy of some DMIs against grey mould in cucumber. Field resistance at one site was associated with the occurrence of isolates with reduced sensitivity, and was attributed to the fact that DMIs had been used previously for control of cucumber powdery mildew at the same site (Elad, 1992). Background sensitivity data are lacking. Hence, one may wonder whether a fast selection of a small subpopulation of the pathogen with low sensitivity to DMIs from the natural population may play a role. Such a process would explain the low number of DMIs registered for grey mould control (De Waard, 1992[b]).

GENETICS OF RESISTANCE TO DMIs

Extensive studies on the genetic basis of resistance to DMIs have been carried out with laboratory-generated mutants of *Aspergillus nidulans* (Van Tuyl, 1977) and *Nectria haematococca* var. *cucurbitae* (Kalamarakis *et al.*, 1991). Genetic analysis of 202 mutants of *A. nidulans* with low levels of resistance to imazalil identified eight different loci allocated to six different linkage groups. Two additional loci conferring imazalil-resistance were identified in cycloheximide-resistant mutants. The mutants showed cross-resistance to fenarimol. These results demonstrate that resistance to DMIs in *A. nidulans* has a polygenic basis. Mutations for resistance to imazalil gave rise to pleiotropic effects such as resistance or hypersensitivity to unrelated toxicants, cold-sensitivity or reduced growth rates (Fuchs and De Waard, 1982). Allelic mutations in the same locus could result in different levels of resistance to imazalil. The effects of the two most frequently identified loci, *imaA* and *imaB*, together with a modifier gene, were additive. Genetic analysis of 51 mutants of *N. haematococca* var. *cucurbitae* with low levels of resistance to fenarimol also identified a polygenic system for resistance with at least nine chromosomal loci involved. The strains showed low levels of cross-resistance to some other DMI fungicides. All mutations appeared to be pleiotropic, having more or less adverse effects on saprophytic fitness and pathogenicity. Analysis of 30 mutants with high resistance to triadimenol only recognized one locus (Kalamarakis *et al.*, 1989). This mutation appeared to be highly mutable and did not lead to the pleiotropic mutations observed with the fenarimol-resistant mutants. The mutants were cross-resistant to other triazole DMIs, but showed no change in sensitivity to imidazole DMIs or even possessed an increased sensitivity. The authors suggest that resistance of *N. haematococca* var. *cucurbitae* is caused by major gene resistance. Genetic evidence, therefore, points to more than one mechanism of resistance operating against DMIs.

Only a few of the pathogens listed in Table 1 have been used to unravel the genetic basis for control of DMI resistance. This is understandable, since many of these pathogens lack a perfect stage or are more difficult to cross than *A. nidulans* and *N. haematococca* var. *cucurbitae*. Analysis of a low level of field resistance of *Venturia inaequalis* to DMIs showed that only a single gene was involved (Stanis and Jones, 1985). A more recent report suggests that resistance to DMIs in *V. inaequalis* is polygenic (Sholberg and Haag, 1993). For *E. graminis* f.sp. *hordei*, conflicting results have been described. Hollomon *et al.* (1984) suggest that a high level of resistance in this pathogen has a polygenic basis, while Brown *et al.* (1992) conclude that alleles at single loci control sensitivity and resistance in crosses of a sensitive isolate with a moderately resistant and a highly resistant isolate. Isolates of *P. teres* resistant to triadimenol may lack clear cross resistance to propiconazole (Peever and Milgroom, 1992). Resistance to triadimenol is conferred by alleles at the same locus (qualitative resistance), while resistance to propiconazole appeared to segregate in a quantitative manner. The variability in correlation coefficients between other DMI-DMI and DMI-fenpropimorph combinations have later been described in more detail

(Peever and Milgroom, 1993). Results indicate that inheritance of DMI resistance may not be the same for different pathogens and DMIs.

It is difficult to conclude whether the polygenic mechanism of resistance shown to be present in laboratory-generated isolates also plays a major role in DMI-resistant field isolates. On one hand, the stepwise development of resistance in *E. graminis* f.sp. *hordei*, *S. fuliginea*, and *V. inaequalis* points to the involvement of more than one gene. On the other hand, genetic analysis of DMI-resistant field isolates identified both monogenic and polygenic resistance. Laboratory-generated mutants often show reduced comparative fitness, and this phenomenon may counteract the development of polygenic resistance development under field conditions. However, loss in comparative fitness may be overcome by continued selection in natural populations for both DMI resistance and normal virulence.

MECHANISM OF NATURAL INSENSITIVITY OR RESISTANCE TO DMIs

A wide variety of mechanisms may operate in natural insensitivity or resistance to DMIs (Köller, 1992; Table 2). Reported mechanisms of natural insensitivity are detoxification (triforine), protonation of imidazole DMIs (imazalil), lack of activation (triadimefon), deposition in cell compartments (triadimenol) and tolerance of toxic sterols. In theory, these mechanisms may also be operative as mechanisms of resistance. However, this has not been observed for any of the DMI-resistant laboratory mutants or field isolates investigated. The mechanisms reported to operate in DMI-resistant laboratory mutants are: increased efflux from mycelium resulting in reduced accumulation, a defect in sterol 14α-demethylation, circumvention of toxic sterol formation, and overproduction of cytochrome-P450-dependent sterol 14α-demethylase ($P450_{14DM}$).

TABLE 2. Mechanisms of natural insensitivity and resistance to DMIs.

Mechanism	Authors
Detoxification	Gastonyi and Josepovits, 1975
Protonation	Siegel *et al.*, 1977
Increased efflux from mycelium	De Waard and Van Nistelrooy, 1979
Lack of activation	Gastonyi and Josepovits, 1979
Defect in sterol 14α-demethylation	Walsh and Sisler, 1982
Circumvention of toxic sterol formation	Taylor *et al.*, 1983
Overproduction of $P450_{14DM}$	Kalb *et al.*, 1986
Deposition in cell compartments	Hippe, 1987
Tolerance of toxic sterols	Weete and Wise, 1987
Induced resistance response	Smith and Köller, 1990
Decreased affinity of $P450_{14DM}$	Vanden Bossche *et al.*, 1990

Increased energy-dependent efflux in resistant mutants counteracts passive influx of DMIs in mycelium and results in a relatively low and constant level of accumulation. This will reduce complex formation between DMIs and their target site, $P450_{14DM}$, and hence may explain the relatively low levels of resistance observed. While efflux in the resistant mutants has a constitutive character, activity in wild-type isolates appears to be inducible, resulting in a

transient accumulation pattern in time (De Waard and Van Nistelrooy, 1979). Fenarimol-efflux activity in wild-type isolates was inducible by pyrimidine, triazole and imidazole DMIs, but not by carbendazim, carboxin, and chloroneb; indicating a specific effect of DMIs (De Waard and Van Nistelrooy, 1981). In both wild-type isolates and resistant mutants, efflux activity could be inhibited by many metabolic inhibitors (De Waard and Van Nistelrooy, 1987). These may be regarded as potential synergists of DMI toxicity (De Waard and Van Nistelrooy, 1984[a]; 1984[b]). Increased energy-dependent efflux has now been described as a mechanism of resistance to various DMIs (De Waard and Van Nistelrooy, 1988) in *Aspergillus nidulans* (De Waard and Van Nistelrooy, 1979), *Penicillium italicum* (De Waard and Van Nistelrooy, 1984[b]), *Candida albicans* (Ryley *et al.*, 1984), *Monilia fructicola* (Ney, 1988), and *N. haematococca* var. *cucurbitae* (Kalamarakis *et al.*, 1991).

A defect in sterol 14α-demethylation and circumvention of toxic sterol formation are common mechanisms of resistance in *Ustilago maydis*, *Candida albicans* and *Saccharomyces cerevisiae*, but do not seem to operate in filamentous fungi. Demethylation defective mutants of *U. maydis* lack ergosterol but contain various C14 sterols (*e.g.* 14α-methylfecosterol) which are probably functional in membranes of the fungus (Walsh and Sisler, 1982). In demethylation-defective mutants of *S. cerevisiae*, a second mutation, a defect in C5-6 desaturation, circumvents the formation of toxic sterols (*e.g.* 14α-methyl-3,6 diol) by which these double mutants become viable (Taylor *et al.*, 1983). Mutants of *U. maydis* and *S. cerevisiae* have a considerably slower growth rate than the corresponding wild-types. Therefore, it is not likely that these mechanisms of resistance will be relevant in field-resistant isolates of plant pathogens. Resistance caused by overproduction of $P450_{14DM}$ has been observed in transformants of *S. cerevisiae* with multiple copies of the $P450_{14DM}$ coding gene (Kalb *et al*, 1986) and by $P450_{14DM}$ gene amplification (Vanden Bossche, pers. comm.). The result is compensation of the target site. It seems possible that these mechanisms may also play a role in decreased DMI sensitivity in plant pathogens.

The molecular mechanism leading to the induced resistance response to triadimenol in *Ustilago avenae* remains to be elucidated (Smith and Köller, 1990). Mechanisms which could be involved are: compensation by increased production of target sites, an altered lipid metabolism, and partitioning of the fungicide in cell compartments such as vacuoles (Hippe, 1987).

The most frequently observed mechanism of resistance to fungicides is decreased affinity of the target site for the fungicide. However, reports on resistance to DMIs caused by decreased affinity of $P450_{14DM}$ to these fungicides, without affecting functioning of the enzyme in sterol biosynthesis, are rare. It has been reported for resistance to some antimycotics in a *C. albicans* isolate, obtained from patients with chronic mucocutaneous candidosis who relapsed after prolonged treatment with ketoconazole (Vanden Bossche *et al.*, 1990). The reason for the reduced virulence of the isolate is not known. The potency of imazalil to inhibit ergosterol biosynthesis in cell-free preparations of laboratory-generated strains of *P. italicum* with different levels of resistance to imazalil is the same (Guan and De Waard, 1993). These results suggest that DMI resistance in this plant pathogen is not based on changes in affinity of $P450_{14DM}$. This mechanism of resistance has not yet been reported for other plant pathogens. The reason for this may be a technical one, since $P450_{14DM}$ is very unstable during preparation of cell-free extracts of filamentous fungi. The availability of proper methods now paves the way to do such experiments (Guan *et al.*, 1992; Stehmann *et al.*, 1994). It would, for instance, be feasible to study the affinity of $P450_{14DM}$ in imazalil-resistant packing-house isolates of *Penicillium digitatum* and in triadimenol-resistant mutants of *N. haematococca* var. *cucurbitae*. The method is not suitable for slow growing fungi or obligate parasites. In these instances a completely different approach, based on cloning and characterization of the sterol 14α-demethylase gene, should be adopted (De Waard, unpublished results; Holloman *et al.*, 1990).

CONCLUDING REMARKS

Extensive efforts have been made to elucidate the mechanism(s) of insensitivity and resistance to DMI fungicides. The fragmentary evidence available so far points to the operation of different mechanisms, and suggests that these mechanisms are not necessarily related to a decrease in affinity of the target site of DMIs, $P450_{14DM}$, in sterol biosynthesis. The variety in mechanisms of insensitivity and resistance also corroborates with the preliminary observations that the genetic basis of resistance may vary for different organisms. Differences in resistance mechanisms may be especially relevant for laboratory-generated mutants of model fungi and field-resistant isolates of plant pathogens, since reductions in comparative fitness or pathogenicity are often restricted to the first category. Much additional knowledge is needed to fully understand the phenomenon.

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SENSITIVITY BEHAVIOUR OF *SEPTORIA TRITICI* POPULATIONS ON WHEAT TO CYPROCONAZOLE

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ABSTRACT

In a sensitivity monitoring program, bulk samples of *Septoria tritici* collected from wheat fields in the UK were tested against cyproconazole and flutriafol. The width of the sensitivity distribution (between lowest and highest EC 50 value) was a factor of about 30 and 60 for cyproconazole and flutriafol, respectively. No shifts in sensitivity were found when the 1992 and the 1993 populations were compared. In the two years mean EC 50 values of the populations were 0.2 mg/l and 0.1 mg/l for cyproconazole and 1.0 mg/l and 0.8 mg/l for flutriafol, respectively. No difference was observed in the sensitivity distributions between samples from DMI-treated fields and fields not treated with DMI fungicides. Sensitivity distributions of samples from different regions were similar and there was no correlation between sensitivity and sampling date. Positive cross resistance between cyproconazole and flutriafol was found for entire populations but also for most individual strains. So far, there is no indication of a decreased sensitivity of this pathogen against cyproconazole.

INTRODUCTION

In northern Europe, *Septoria tritici* (teleomorph *Mycosphaerella graminicola*), is one of the most important pathogens of wheat, causing leaf blotch on many cultivars. Since the control of this pathogen cannot easily be achieved by breeding programs, the use of pesticides has been common practice for many years. Amongst them, broad spectrum fungicides of the DMI class (Demethylation Inhibitors), mainly triazoles, are used successfully on a large acreage. The development of resistance to triazoles is known to have occurred in *Erysiphe gra/minis* and is also a potential risk with *S. tritici*. Therefore, a sensitivity monitoring was initiated two years ago for *S. tritici*. The objectives of the program were to evaluate the variability in triazole-sensitivity among field isolates (bulk samples) of *S. tritici* from different regions of the UK, and to compare isolates collected in fields treated or not treated with DMI's. In addition, a comparison of the sensitivity distribution was carried out between the years 1992 and 1993 for both cyproconazole and flutriafol.

MATERIALS AND METHODS

Leaf samples, infected with *S. tritici*, were collected between the end of May and beginning of August in different regions of England and Scotland. They were surface-sterilized with 2 % sodium hypochlorite solution, placed on water agar and exposed to UV light to

stimulate pycnidia production. A spore suspension (bulk samples) was transferred to potato dextrose agar (PDA) agar containing 100 mg/l streptomycin and 100 mg/l rifampicin. After 2 days the fungus was transferred to malt yeast agar and incubated at 20° C until sporulation occurred. A spore suspension (10^4/ml) was spread on the surface of PDA-plates containing cyproconazole or flutriafol at concentrations of 0.005, 0.05, 0.5, 5, and 50 mg AI/l. After incubation for 6 days at 20° C, growth and sporulation of the isolates were assessed visually and compared to that on unamended agar. Dose-response correlations were used to calculate EC 50 values (effective concentrations resulting in 50% inhibition of fungal growth).

RESULTS AND DISCUSSION

Sensitivity distributions in 1992 and 1993

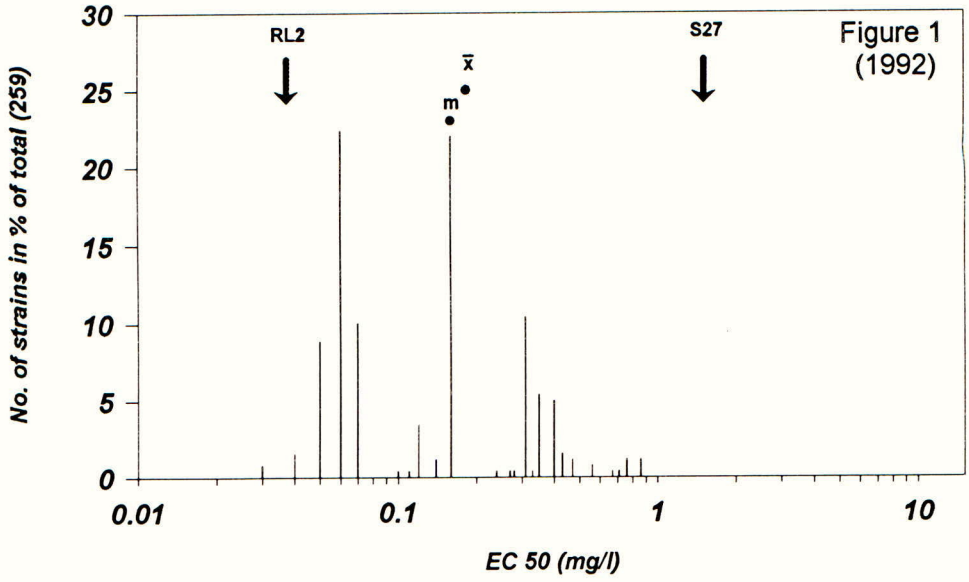
The EC 50 values for cyproconazole of the strains isolated in 1992 ranged from 0.03 to 0.9 mg/l, the average EC 50 was 0.2 mg/l. In 1993, the values ranged between 0.01 and 0.4 mg/l with an average EC 50 of 0.15 mg/l (Figs. 1 and 2). With flutriafol, the EC 50 values varied from 0.1 to 6.3 mg/l in 1992 (average 1.0 mg/l). In 1993, the EC 50 values ranged between 0.04 and 3.2 mg/l; the average EC 50 was 0.8 mg/l (Figs. 3 and 4). The width of the sensitivity distribution (between the lowest and highest EC 50 values) in both years was about the same (cyproconazole: factor of 30 in 1992, factor of 40 in 1993; flutriafol: factor of 60 in 1992, factor of 80 in 1993). The 1993 populations were somewhat more sensitive than those from 1992, but also the reference strains were more sensitive. The difference in sensitivity could partly be explained by slight changes in the testing method. No strain was found with a sensitivity level as low as that of the less sensitive reference strain S27. However, some strains proved to be even more sensitive than the sensitive reference strain RL2.

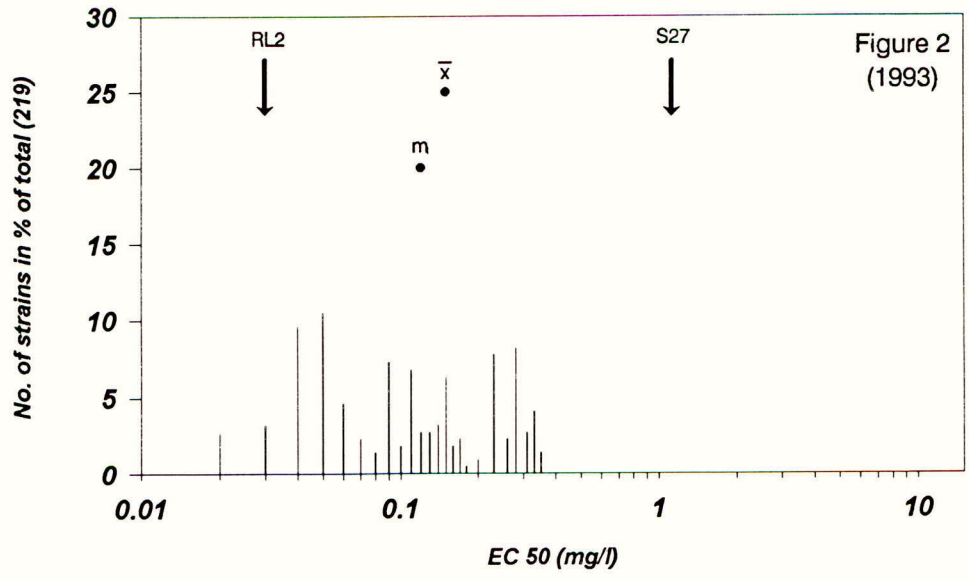
When the sensitivity distributions to flutriafol and cyproconazole of all samples were compared, flutriafol was about 6 to 10 times (1993 and 1992 figures) less active than cyproconazole. With individual strains, the difference in sensitivity to the two tested triazoles, expressed by dividing the EC 50 values of flutriafol and cyproconazole, varied by a factor of 1 to 34 (in 1993) and 1 to 105 (in 1992). Strains of the 1992 population with the most extreme EC 50 values were analysed, i.e. 5 % of the total population both at the upper and the lower end: The flutriafol/cyproconazole EC50-factor was between 1 and 15 (Table 1). Only 6 strains out of 252 resulted in factors higher than 50 (extreme: 105) and 16 strains had factors lower than 2 (extreme: 1.0). Therefore, a good positive cross resistance pattern between the two tested triazole fungicides was found at the population level and also for most of the individual strains. These results show clearly, how dangerous it is to do cross resistance studies with only a few strains.

Samples collected at different sites

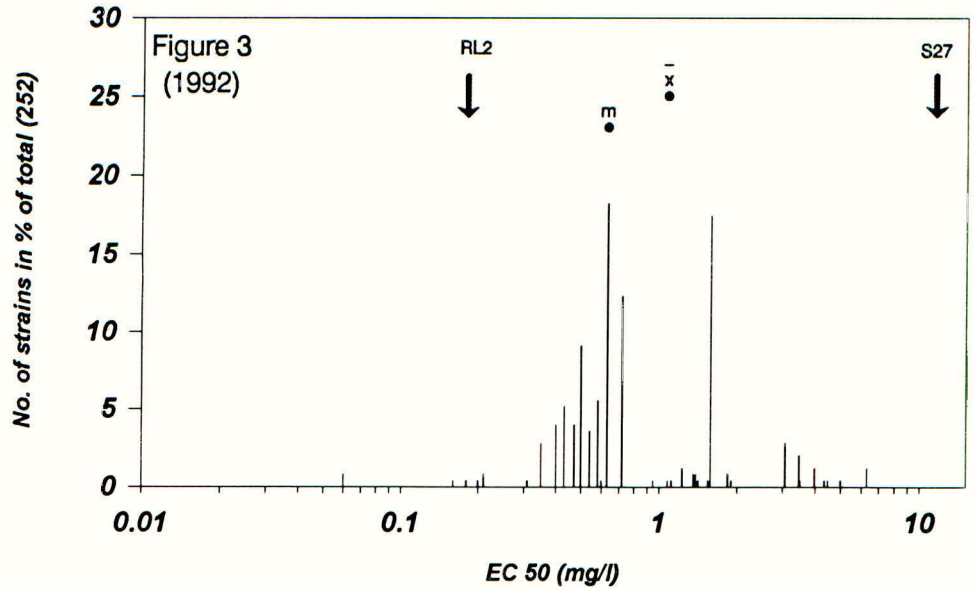
No difference was observed in the sensitivity distribution of samples isolated in both years from fields that had not been treated with DMI-fungicides (Fig. 5) and from DMI-treated fields (Fig. 6). Comparing the sensitivity distribution of 1992 with 1993, and of DMI-treated with -untreated populations, no differences were detected by the non-parametric Kolmogoroff-Smirnoff test. The EC 50 values (1993) found with samples from untreated fields ranged from

FIGS. 1 and 2: Sensitivity distribution of bulk samples of *S. tritici* to cyproconazole, isolated from wheat fields in the UK in 1992 (Fig. 1) and in 1993 (Fig. 2). RL2 and S27 are sensitive and less sensitive reference strains, respectively; $\bar{x}$ and m are mean and median of EC50 value distribution





FIGS. 3 and 4: Sensitivity distribution of bulk samples of *S. tritici* to flutriafol isolated from wheat fields in the UK in 1992 (Fig. 3) and in 1993 (fig 4). (Symbol explanation see Fig. 1)



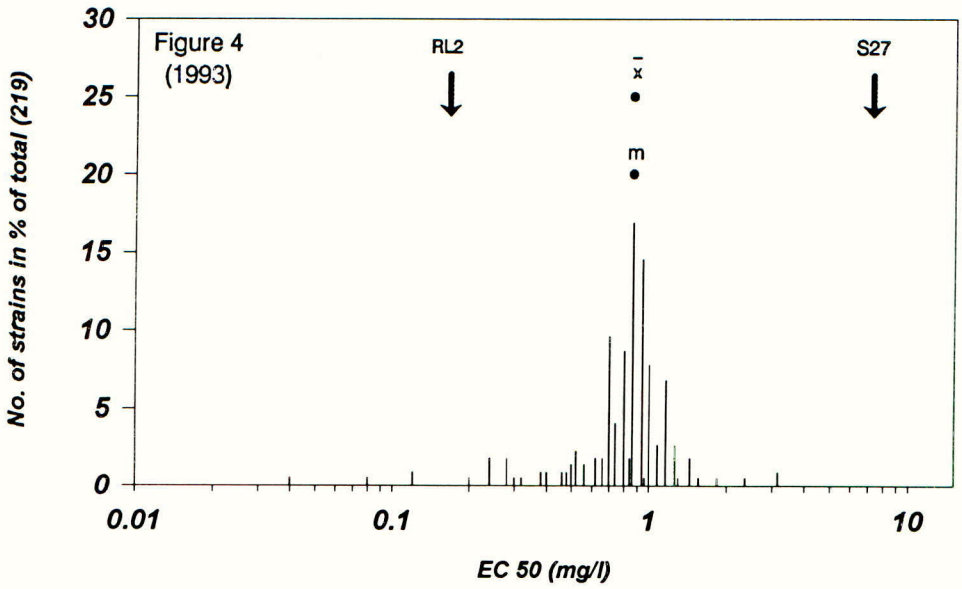


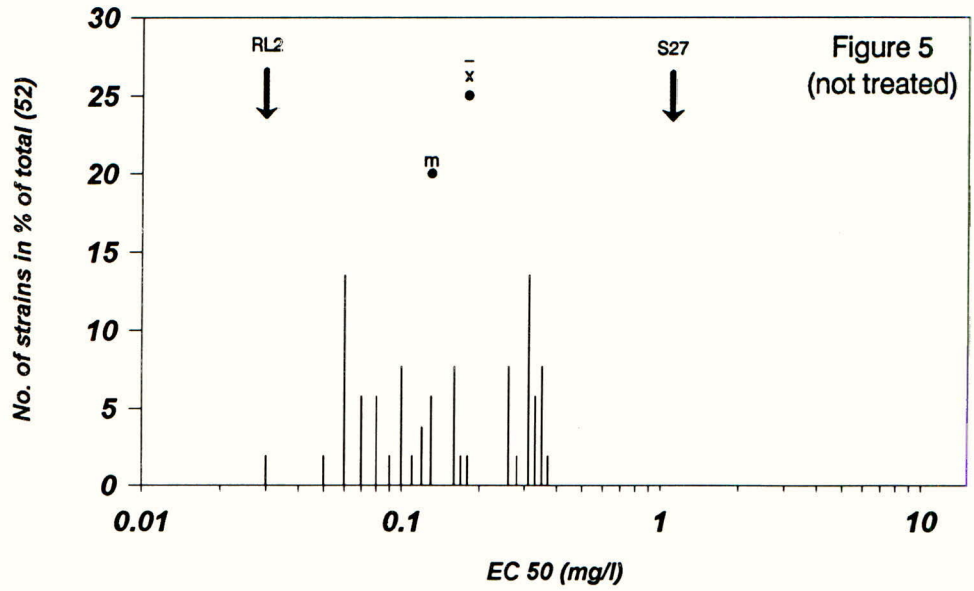
TABLE 1: Sensitivity of individual strains of *S. tritici* to cyproconazole and flutriafol *in vitro*

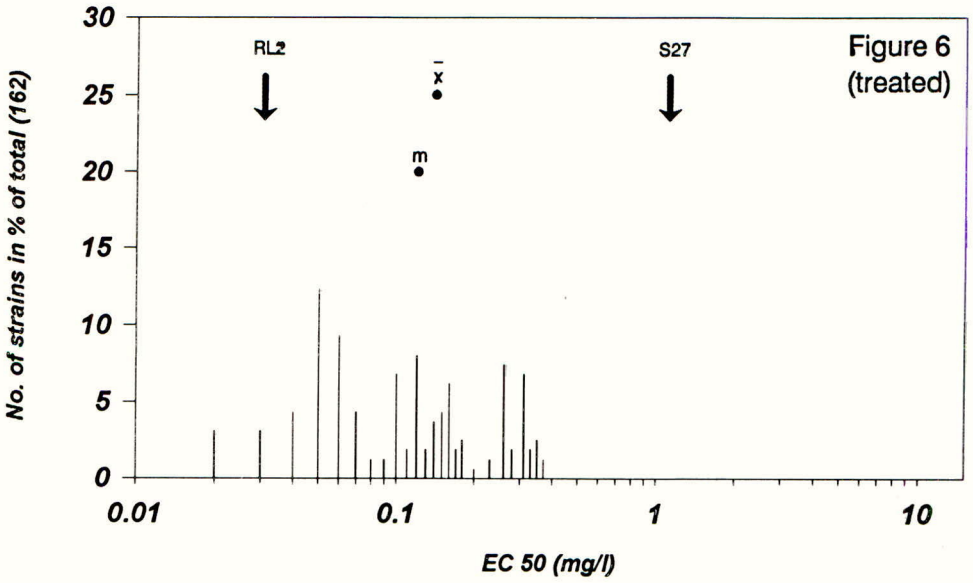
strain no.	sensitivity (EC 50 in mg/l) to cyproconazole	flutriafol	factor [a)] (EC 50 flu/EC 50 cyp)
highly sensitive to cyproconazole:			
183	0.03	0.4	13
28	0.04	0.4	10
176	0.04	0.4	10
55	0.04	0.4	10
203	0.04	0.6	15
244	0.05	0.2	4
134	0.05	0.4	8
222	0.05	0.4	8
151	0.05	0.4	8
139	0.05	0.4	8
81	0.05	0.4	8
177	0.05	0.4	8
95	0.05	0.5	10
less sensitive to cyproconazole:			
36	0.43	3.5	8
16	0.47	0.6	1
30	0.47	1.4	3
245	0.47	1.6	3
121	0.56	1.2	2
140	0.56	1.4	2
192	0.67	3.1	5
116	0.71	1.8	3
172	0.76	1.6	2
133	0.76	1.8	2
117	0.86	1.4	2
126	0.86	1.6	2
125	0.86	1.6	2

[a)] A factor of 5 should be considered as variation among tests (a total of 252 strains have been tested in 12 tests). This factor is based on the EC 50 values found for the reference strains in individual tests. In addition, a factor of 6 - 10 represents the difference in intrinsic activity between cyproconazole and flutriafol. Therefore, strains with factors (EC 50 flu/EC 50 cyp) between 2 and 50 are considered to represent positive cross resistance.

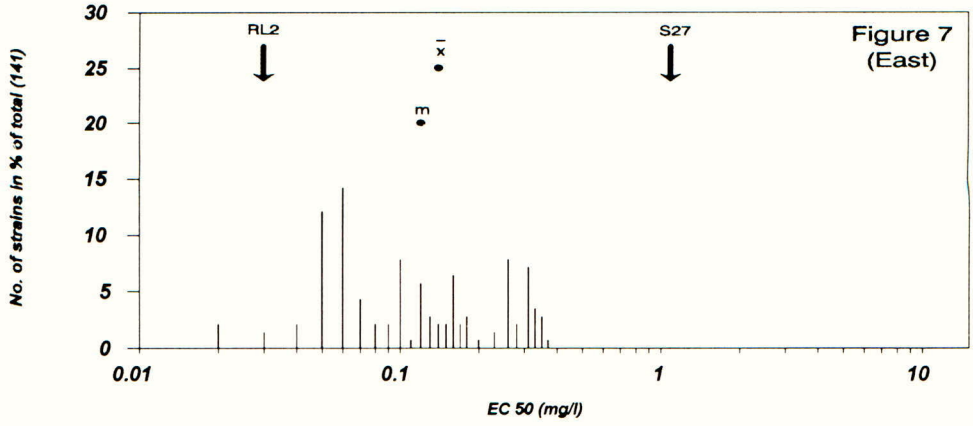
0.03 to 0.4 mg/l (average 0.18 mg/l). The values of samples from DMI-treated fields varied between 0.01 and 0.4 mg/l (average 0.14 mg/l). Three regions have been analyzed in more detail: East Anglia/Midlands (141 samples, Fig. 7), South/South West (37 samples, Fig. 8) and North/Scotland (41 samples, Fig. 9). There was no obvious difference in the sensitivity distributions of samples from the three regions. The average EC 50 values of the samples from the regions were: East Anglia/Midlands: 0.14 mg/l, South/SouthWest: 0.18 mg/l, North/ Scotland: 0.14 mg/l. The strains from East Anglia/Midlands were sampled over a period of approx. 11 weeks (from the end of May to the beginnig of August). A correlation was

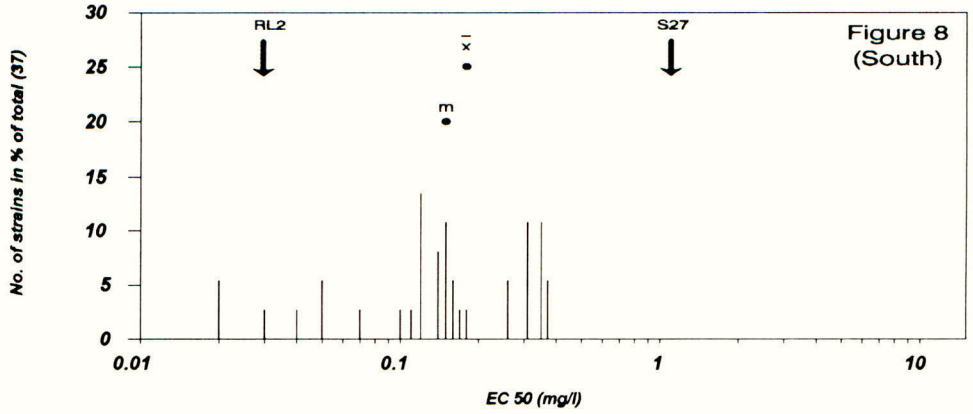
FIGS 5 and 6: Sensitivity distribution of bulk samples of *S. tritici* to cyproconazole, isolated in 1993 in the UK from wheat fields not treated with DMI fungicides (Fig. 5) and treated with DMI fungicides (Fig. 6). (Symbol explanation see Fig. 1)

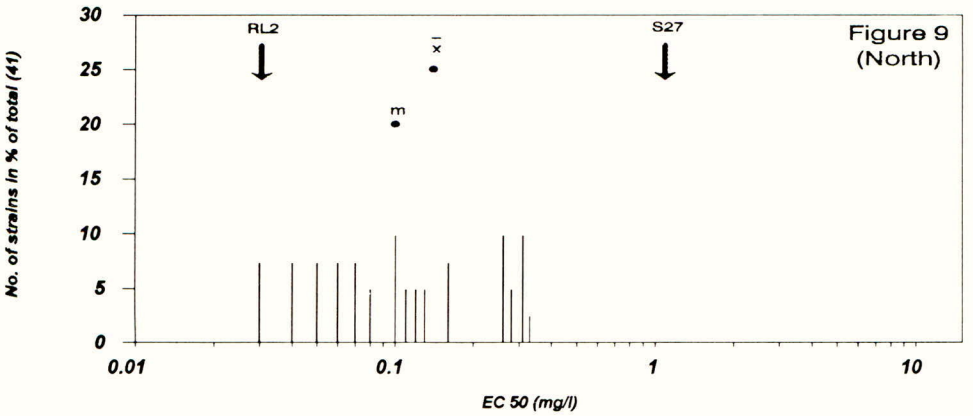




FIGS. 7, 8 and 9: Sensitivity distribution of bulk samples of *S. tritici* to cyproconazole, isolated in 1993 from wheat fields in East Anglia/Midlands (Fig. 7), in the South/South West (Fig. 8) and in North England/Scotland (Fig. 9) (Symbol explanation see Fig. 1)

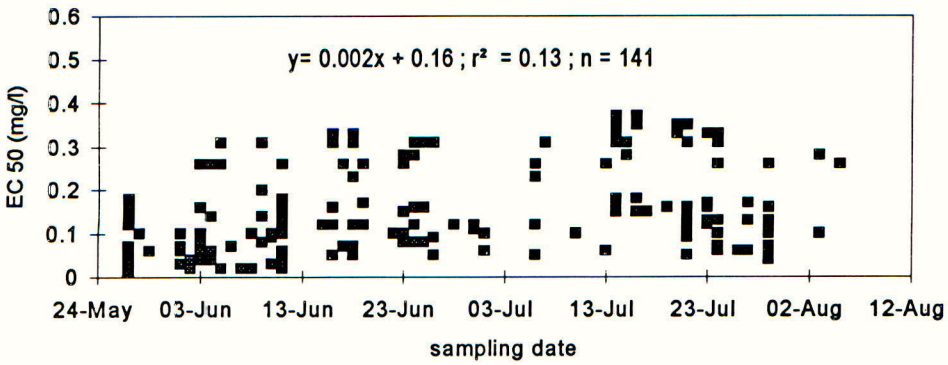






calculated to show whether certain sensitivity levels would dominate throughout the season (Fig. 10). No increase or decrease in sensitivity levels throughout the season was found, i.e. no correlation between the EC 50 values and the sampling date was observed. The highest EC values were found in samples taken in the middle of July.

FIG. 10: Sensitivity of *S. tritici* to cyproconazole, isolated from wheat fields in 1993 in East Anglia/Midlands as a function of the sampling date



CONCLUSIONS

Comparing the two years 1992 and 1993, there is no indication of changes in sensitivity of *S. tritici* populations towards cyproconazole and flutriafol. Because there are no base line data available from years prior to the extensive use of triazole fungicides, it is difficult to conclude whether a sensitivity shift has already occurred and stabilized on a lower sensitivity level (as in powdery mildew of cereals) or, more likely, whether such a shift has not yet occurred, as with rusts of wheat (Ohl and Gisi, this volume). The years to come will reveal whether a sensitivity shift will occur with this pathogen. So far, effective triazole fungicides control the disease caused by *S. tritici* if they are used according to the recommendations.

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