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Changes during a three-year period in the sensitivity to ergosterol biosynthesis inhibitors of Sphaerotheca fuliginea in the Netherlands

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Abstract

Isolates of *Sphaerotheca fuliginea* collected in 1981-1984 in cucumber glasshouses in the Netherlands were tested for their sensitivity to the ergosterol biosynthesis inhibitors (EBIs) fenarimol and imazalil.

The data collected in the 1981 survey indicated that sensitivity to EBIs was lower than that of reference isolates. In the 1982 and 1983 surveys, sensitivity to EBIs continued to decrease. In 1984, data were collected until July; no significant difference in sensitivity with the 1983 level was apparent.

Isolates collected in the district of Limburg, where EBIs were applied less frequently than in the district of Pijnacker, showed a significantly higher sensitivity to EBIs than isolates collected in Pijnacker.

Besides differences in sensitivity to EBIs between years, differences within years were noticed. In 1983, the sensitivity to fenarimol and imazalil was significantly higher in the beginning of the growing season, before any fungicides were applied, than later in the growing season.

Generally, changes in sensitivity to EBIs did not result in full failure of control. In most cases, a change to shorter spray intervals has been sufficient to compensate for the decrease in sensitivity and to achieve proper control by fenarimol and imazalil. The results once more emphasize the necessity of designing strategies to prevent resistance to EBIs.

Additional keywords: cucumber powdery mildew, fungicide resistance, fenarimol, imazalil.

Introduction

The ergosterol biosynthesis inhibitors (EBIs) constitute an important group of systemic fungicides for the control of powdery mildews. Most EBIs interfere with the biosynthesis of ergosterol by inhibiting C-14 demethylation. In view of the specificity of this mechanism of action, it was not surprising that resistance to EBIs was found in vitro (cf. Fuchs and De Waard, 1982). Considering the low level of resistance and the decreased fitness of resistant strains, it was thought unlikely that resistance to EBIs would develop in practice (Fuchs and Drandarevski, 1976). Although indeed EBIs do not readily cause resistance to develop, prolonged and intensive use of EBIs in practice yet might lead to development of resistance (De Waard and Fuchs, 1982).

For the past four years control of cucumber powdery mildew *(Sphaerotheca fuliginea)* has been carried out almost exclusively with the EBIs bitertanol, fenarimol,

imazalil and triforine. Their frequent use, and the observation that this pathogen seems to develop resistance to fungicides fairly easily (Bent et al., 1971; Kooistra et al., 1972; Dekker and Gielink, 1979), made it important to monitor the sensitivity of the fungus to EBIs. In the present study, sensitivity of *S. fuliginea* to EBIs was followed by using a leaf disc test. The survey covered the years 1981 to 1984.

Materials and methods

Plants. Cucumber plants *(Cucumis sativus* L.) cv. Lange Gele Tros were used in the experiments. Growth conditions were as described previously (Schepers, 1984a).

Chemicals. Bitertanol (technically pure) was generously supplied by Bayer A.G., Leverkusen, Fed. Rep. Germany; buthiobate as 10% EC Denmert by AAgrunol B.V., Groningen, the Netherlands; fenarimol (technically pure) by Lilly Research Centre Ltd., Surrey, England; fenpropimorph (technically pure) by Dr R. Maag A.G., Dielsdorf, Switzerland and imazalil (technically pure) by Janssen Pharmaceutica, Beerse, Belgium. Stock solutions were made in methanol. They were stored at −20 °C and freshly prepared every four weeks.

Sampling. Isolates of *S. fuliginea* were sampled by collecting several diseased cucumber leaves from small areas (1 to 2 m^2) in commercial glasshouses. In October 1981, isolates were collected in 50 glasshouses distributed throughout the Netherlands. In 1982, 1983 and 1984 isolates were obtained from 13 glasshouses. Five were situated in the district of Pijnacker, which is the main cucumber growing area of the Netherlands. Four were in the district of Limburg. The remaining four glasshouses in the survey were situated in Breda (district West-Brabant), Vleuten (district Vleuten) and Klazienaveen (district Drenthe) (Schepers, 1984b). All fungicide applications used to control *S. fuliginea* in these glasshouses were recorded. In 1982 and 1983, the mildew population in these glasshouses was sampled three times per season, viz. before the first fungicide treatment (January to May = period A), in July (period B) and in October (period C). In 1984, isolates were collected until July. The number of isolates collected per sampling period in any glasshouse varied from one to six.

Reference isolates were described previously (Schepers, 1985). Isolates originating from foreign countries were received by mail. This import was licensed by the Plant Protection Service in Wageningen, under the restriction that isolates were destroyed after testing.

Preparation of inoculum. Isolates were transferred to mildew-free cucumber plants and incubated under greenhouse conditions (15-25 °C, 60-80% rh) for 7 to 14 days. In this way, inoculum was multiplied, interference with fungicide residues was avoided, and variability in inoculum quality was reduced. Cross-contamination among isolates was avoided by incubating these in separate greenhouse compartments. When many isolates were collected in one period, some were stored on cucumber leaves floating on tap-water in Petri dishes (ø 14 cm) in a growth chamber at 20 °C. These isolates were transferred to fresh leaves every two weeks, until transfer to mildew-free cucumber plants in the greenhouse.

Sensitivity tests. The leaf disc test used to assess sensitivity to EBIs was described previously (Schepers, 1984a).

Results

Fungicide use. The number of fungicide treatments in the glasshouses under survey are presented in Table 1. As fenarimol became available for control of *S. fuliginea* in the summer of 1981, the EBIs used in 1981 were triforine and imazalil only. These chemicals were introduced in 1972 and 1977, respectively. In the district of Limburg the ratio between the number of treatments with EBIs and non-EBIs (Q value) was 0.5. Dinocap was the most frequently used non-EBI fungicide. In the district of Pijnacker in 1982 and 1983, EBIs were used almost exclusively. Bitertanol was introduced in April 1983. However, fenarimol and imazalil remained the most frequently used EBIs. The Q values of Pijnacker for 1982, 1983 and 1984, being 29.0, 10.7 and 3.2 respectively, clearly show the tendency towards the use of more non-EBIs. Bupirimate, dinocap and tolylfluanid predominated among the non-EBIs.

Sensitivity to EBIs. The sensitivity to fenarimol and imazalil of the isolates collected in the survey is given in Tables 2 and 3.

Comparison between years. The data (Tables 2 and 3) and their presentation in cumulative frequency curves (Fig. 1) show that the isolates collected in 1981 had a significantly lower sensitivity to fenarimol and imazalil than the reference isolates. In 1982, the cumulative frequency curves for the sensitivity to fenarimol and imazalil were significantly different from those in 1981. Particularly the lower number of isolates with EC_{50} values for fenarimol between 4×10^{-8} and 8×10^{-8} M and for imazalil between 2×10^{-7} and 8×10^{-7} M contributed to this difference. In 1983,

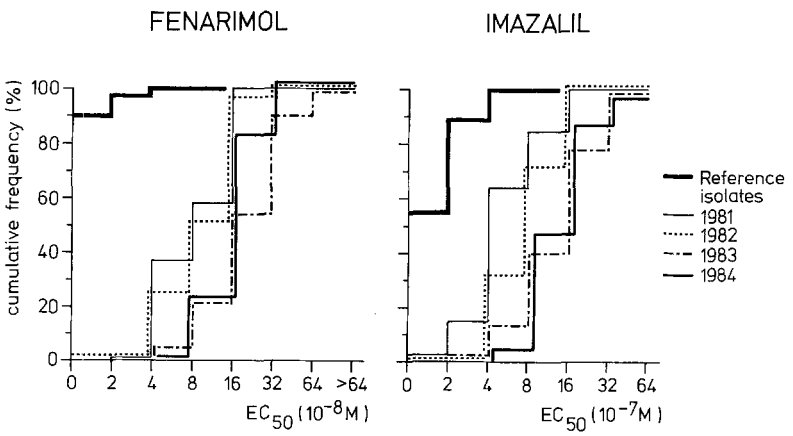

Fig. 1. Cumulative frequency curves of the sensitivity to fenarimol and imazalil of *Sphaerotheca fuliginea* isolates collected from 1981 to 1984 in the Netherlands. The number of isolates tested was 97 in 1981, 75 in 1982, 125 in 1983, and 96 in 1984. During the survey, eight different reference isolates were tested 54 times. All curves were significantly different from each other, except the curves for imazalil of 1983 and 1984 (Kolgomorov-Smirnov, $p = 0.05$).

Table 1. Mean number of treatments with EBIs and other fungicides (non-EBIs) to control *Sphaerotheca fuliginea* in glasshouses in the Netherlands.

Location	1981				1982				1983				1984[5]			
	n[1]	EBIs[2]	non-EBIs[3]	Q[4]	n	EBIs	non-EBIs	Q	n	EBIs	non-EBIs	Q	n	EBIs	non-EBIs	Q
Pijnacker	15	6.3	1.6	3.9	5	11.6	0.4	29.0	5	12.8	1.2	10.7	5	5.8	1.8	3.2
Limburg	11	2.4	4.4	0.5	4	3.7	5.5	0.7	4	7.7	3.7	2.1	4	2.7	2.2	1.2
Vleuten, Breda, Klazienaveen	17	5.3	2.3	2.3	4	10.0	1.7	5.7	4	7.7	6.0	1.3	4	2.7	2.5	1.1
Total	43	4.9	2.6	1.9	13	8.7	2.4	3.6	13	9.7	3.5	2.8	13	3.9	2.1	1.8

[1] Number of glasshouses.
[2] EBIs: bitertanol, fenarimol, imazalil, triforine.
[3] Non-EBIs: dinocap, ditalimfos, bupirimate, pyrazophos, tolylfluanid.
[4] Q = ratio between number of treatments with EBIs and non-EBIs.
[5] Data from samples collected until July.

the numbers of isolates with EC_{50} values for fenarimol higher than 32×10^{-8} M and for imazalil between 16×10^{-7} and 64×10^{-7}M were significantly higher than in 1982.

In 1984, when isolates were collected until July, fewer isolates were observed with EC_{50} values higher than 32×10^{-8} M for fenarimol. The cumulative frequency curve for the sensitivity to imazalil was not significantly different from that in 1983.

Comparison within years. The cumulative frequency curves of the sensitivity to fenarimol and imazalil in period A of 1983 were significantly different from those in periods B and C (Fig. 2). Between periods B and C of 1983 no significant differences were noticed. In 1982, the only significant difference was observed between the sensitivity to fenarimol in periods A and C.

Comparison between districts. When the EBI sensitivities of all isolates collected in the various districts from 1981 to 1984 are presented in cumulative frequency curves, the isolates from the district of Limburg showed a significantly higher sensitivity to fenarimol and imazalil than those from the district of Pijnacker (Fig. 3). The isolates from Breda, Vleuten and Klazienaveen showed an intermediate sensitivity to fenarimol, while their sensitivity to imazalil was similar to that of the isolates from Pijnacker.

Case study. In one particular glasshouse in Pijnacker, in which each year a cucumber crop was maintained from December to October, the sensitivity of *S. fuliginea* to EBIs was followed from 1981 to 1984. In 1981, ten EBI treatments were used to successfully control cucumber powdery mildew. In 1982, this regime was maintained without problems. In 1983, the first mildew infection was observed two weeks after planting, in the beginning of January. Treatments were applied every 10 days, alternating triforine with imazalil. Until May 1983, 12 EBI treatments had been applied, which did not give the expected control. Triforine especially seemed to have lost its efficacy (Schepers, 1983). At that time the cucumber crop was heavily infected by

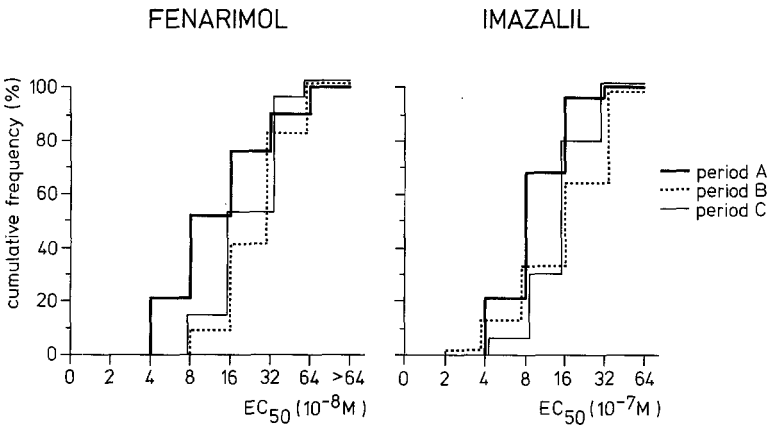

Fig. 2. Cumulative frequency curves of the sensitivity to fenarimol and imazalil of *Sphaerotheca fuliginea* isolates collected in three periods of the growing season of 1983. The number of isolates collected in period A was 28, in period B 45, and in period C 50. The curves for period A differed significantly from the curves of periods B and C (Kolgomorov-Smirnov, p = 0.05).

Table 2. Sensitivity to fenarimol of *Sphaerotheca fuliginea* isolates collected from 1981 to 1984 in the Netherlands.

Location		Number of isolates in each EC_{50} category																											
		0-2[1]				2-4				4-8				8-16				16-32				32-64				>64			
		81[2]	82	83	84	81	82	83	84	81	82	83	84	81	82	83	84	81	82	83	84	81	82	83	84	81	82	83	84
Pijnacker	A[3]	—	0[4]	0	0	—	0	0	0	—	5	1	0	—	4	5	2	—	8	3	10	—	0	3	1	—	0	3	0
	B	—	0	0	0	—	0	0	0	—	0	0	0	—	1	1	5	—	6	3	9	—	0	11	3	—	0	2	0
	C	0	0	0	—	0	0	0	—	5	0	0	—	6	1	0	—	31	5	2	—	0	2	16	—	0	0	1	—
Limburg	A	—	0	0	0	—	0	0	0	—	7	1	2	—	1	2	9	—	0	2	2	—	0	1	0	—	0	0	0
	B	—	1	0	0	—	0	0	0	—	1	0	0	—	1	3	1	—	1	10	15	—	0	1	2	—	0	0	0
	C	0	0	0	—	0	0	0	—	10	0	0	—	6	3	6	—	3	5	9	—	0	0	0	—	0	0	0	—
Vleuten, Breda, Klazienaveen	A	—	0	0	0	—	0	0	0	—	2	4	0	—	5	2	0	—	1	2	9	—	0	0	8	—	0	0	0
	B	—	0	0	0	—	0	0	0	—	3	0	0	—	2	0	4	—	2	2	13	—	0	7	2	—	0	6	0
	C	0	0	0	—	1	0	0	—	20	0	0	—	8	1	1	—	7	7	9	—	0	0	6	—	0	0	1	—
Total		0	1	0	0	1	0	0	0	35	18	6	2	20	19	20	21	41	35	42	58	0	2	45	16	0	0	13	0
Reference isolates[5]			49				4				1				0				0				0				0		

[1] EC_{50} values in 10^{-8} M.
[2] Year of survey.
[3] A: collected in February to May; B: collected in July; C: collected in October.
[4] Number of isolates. —: no isolates collected.
[5] From 1981 of 1984 eight different reference isolates were tested 54 times.

Table 3. Sensitivity to imazalil of *Sphaerotheca fuliginea* isolates collected from 1981 to 1984 in the Netherlands.

Location		Number of isolates in each EC_{50} category																			
		0-2[1]				2-4				4-8				8-16				16-32				32-64			
		81[2]	82	83	84	81	82	83	84	81	82	83	84	81	82	83	84	81	82	83	84	81	82	83	84
Pijnacker	A[3]	—	0[4]	0	0	—	0	0	0	—	6	3	1	—	9	3	6	—	2	7	3	—	0	2	1
	B	—	0	0	0	—	0	0	0	—	0	1	0	—	2	3	12	—	5	5	4	—	0	8	1
	C	0	0	0	—	1	0	0	—	20	1	0	—	11	3	0	—	10	4	12	—	0	0	7	—
Limburg	A	—	1	0	0	—	0	0	0	—	7	1	2	—	0	5	8	—	0	0	3	—	0	0	0
	B	—	0	0	0	—	0	1	0	—	4	4	1	—	0	6	8	—	0	2	9	—	0	0	0
	C	1	0	0	—	8	0	0	—	8	1	3	—	0	5	8	—	2	2	4	—	0	0	0	—
Vleuten, Breda, Klazienaveen	A	—	0	0	0	—	0	1	0	—	3	2	0	—	4	5	0	—	1	0	9	—	0	0	8
	B	—	0	0	0	—	0	0	0	—	0	0	2	—	5	0	6	—	2	7	9	—	0	8	2
	C	1	0	0	—	4	0	0	—	19	1	0	—	9	2	4	—	3	5	9	—	0	0	3	—
Total		2	1	0	0	13	0	2	0	47	23	14	6	20	30	34	40	15	21	46	37	0	0	28	12
Reference isolates[5]		30				18				6				0				0				0			

[1] EC_{50} values in 10^{-7} M.
[2] Year of survey.
[3] A: collected in February to May; B: collected in July; C: collected in October.
[4] Number of isolates. —: no isolates collected.
[5] From 1981 of 1984 eight different reference isolates were tested 54 times.

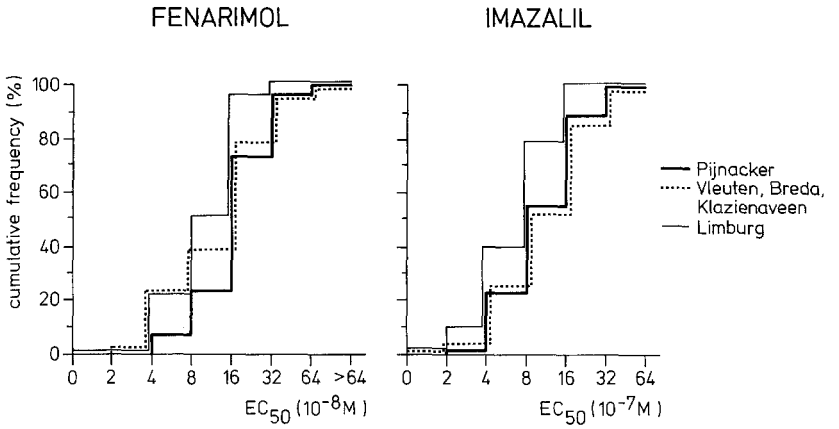

Fig. 3. Cumulative frequency curves of the sensitivity to fenarimol and imazalil of *Sphaerotheca fuliginea* isolates collected in various districts of the Netherlands. The number of isolates collected from 1981 to 1984 in Pijnacker was 154, in Limburg 104, and in Breda, Vleuten and Klazienaveen 134. The curves for fenarimol and imazalil for isolates from Pijnacker and Limburg were significantly different. The curves for imazalil for isolates from Limburg and Breda, Vleuten and Klazienaveen were significantly different (Kolgomorov-Smirnov, $p = 0.05$).

S. fuliginea. The isolates showed a low sensitivity to fenarimol (Table 4). EC_{50} values for fenarimol of over 64×10^{-8} M were found. The advice to use non-EBIs was followed. It resulted in considerable reduction of the mildew infection. From June to October, eight EBI treatments were sufficient to control the disease satisfactorily. In 1984, mildew appeared much later, in April. The first two treatments were carried out with dinocap and tolylfluanid, respectively. Thereafter, imazalil (4 ×) and fenarimol (1 ×) were applied successfully until July. Isolates collected in 1984 did not show the low sensitivity to fenarimol as observed in May 1983.

Table 4. Number of treatments with fungicides in a glasshouse in Pijnacker and the sensitivity of isolates of *Sphaerotheca fuliginea* from that glasshouse to fenarimol.

Year	Number of isolates in each EC_{50} category[1]					Number of sprays	
	4-8	8-16	16-32	32-64	>64	EBIs	non-EBIs
1981	0	0	1	0	0	10	0
1982	2	1	4	1	0	9	1
1983	0	0	2	12	5	20	4
1984[2]	0	4	4	0	0	5	2

[1] EC_{50} values in 10^{-8} M.
[2] Data excluding period C (October).

Table 5. Toxicity of EBIs to various isolates of *Sphaerotheca fuliginea* in leaf disc tests.

Isolate[1]	EC_{50} (10^{-7} M)				
	bitertanol	buthiobate	fenarimol	fenpropimorph	imazalil
S1	0.6	5.5	0.1	17.0	0.6
S2	1.0	6.0	0.2	30.0	3.0
S3	1.7	–[2]	0.1	30.0	1.0
R1	10.0	300.0	4.5	21.0	45.0
R2	10.0	300.0	5.0	30.0	45.0
R3	17.0	–	10.0	10.0	30.0

1 Isolates S1, S2 and S3 are reference isolates; isolates R1, R2 and R3 were collected from glasshouses in the Netherlands in 1982.
2 Not determined.

Cross-resistance. Positively correlated cross-resistance in isolates collected in the Netherlands was observed for bitertanol, buthiobate, fenarimol and imazalil (Table 5). No cross-resistance was observed for fenpropimorph.

Mail survey. All isolates received were identified as *S. fuliginea.* Isolates originating from countries where EBIs have never been used, showed a sensitivity to fenarimol and imazalil similar to that of the reference isolates (Table 6). Most of the isolates that originated from EBI-treated crops were less sensitive to fenarimol and imazalil than the reference isolates.

Table 6. Sensitivity to fenarimol and imazalil of *Sphaerotheca fuliginea* isolates from countries with various spray regimes.

Spray regime	Fungicide tested	Number of isolates in each EC_{50} category						
		0-2[3]	2-4	4-8	8-16	16-32	32-64	>64
Including EBIs[1]	fenarimol	7	1	2	2	13	15	6
	imazalil	3	1	6	6	13	6	0
Excluding EBIs[2]	fenarimol	11	0	1	0	0	0	0
	imazalil	5	2	0	0	0	0	0
Reference isolates	fenarimol	49	4	1	0	0	0	0
	imazalil	30	18	6	0	0	0	0

1 Isolates controlled with EBIs originated from: England, Greece, Israel, Jordan, Norway, Spain and Switzerland.
2 Isolates not controlled with EBIs originated from: Canada, England, Israel, Japan, South-Africa and USA.
3 EC_{50} values for fenarimol in 10^{-8} M and for imazalil in 10^{-7} M.

Discussion

The sensitivity of *S. fuliginea* to fenarimol and imazalil, which in 1981 was already lower than that of the reference isolates, continued to decrease in 1982 and 1983. In 1984, this development seemed to have slowed down. The decreased sensitivity of *S. fuliginea* in 1981 may have been 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 fact may explain the further increase in number of isolates with decreased sensitivity to fenarimol and imazalil in 1982 and 1983. The large number of isolates collected in October 1981 necessitated more transfers to fungicide-free leaves than in the other years. This might have increased the sensitivity to EBIs somewhat. However, there are indications that the effect of subculturing on sensitivity to EBIs is only apparent over a longer period of time (Schepers, 1985).

The frequency of treatment with EBIs, determined by the severity of the mildew epidemic and by regional differences in the advice by extension officers, varied between years and between districts (Table 1). In Pijnacker, and to a lesser degree in Klazienaveen, where many cucumber crops are present in a small area, the infection pressure is usually higher than in other districts (Schepers, 1984b). EBIs with curative and systemic properties were applied more often in these districts than elsewhere. In 1982 and 1983, for example, mildew infections appeared early. They were difficult to control; 10 to 20 EBI treatments were no exception in Pijnacker and Klazienaveen. In 1983, this frequent use of EBIs resulted in the occurrence of many isolates with high EC_{50} values (Tables 2 and 3) and in at least one case of failure of disease control. A correlation between high EC_{50} values of EBIs in leaf disc tests and failure of disease control in foliar spray tests was demonstrated earlier (Schepers, 1983). Triforine especially showed such a low efficacy in controlling glasshouse isolates, that under high disease pressure no control could be achieved with this fungicide. Failure of disease control due to decreased sensitivity of cucumber powdery mildew to EBIs in other countries has been described by Huggenberger et al. (1984). The isolates received by mail from foreign countries showed that application of EBIs causes changes in sensitivity to EBIs of *S. fuliginea* populations (Table 6).

In the district of Limburg, where substantially fewer EBIs were used during the survey period, the sensitivity of the cucumber powdery mildew population changed significantly more slowly than in the district of Pijnacker (Fig. 3). The late start of the mildew epidemics in Limburg, an emphasis placed on alternation with non-EBIs, and the fact that the majority of the growers include a cucumber-free period during August, may have contributed to the smaller amounts of EBIs used in this district. In England and Scotland the use of EBIs influenced the degree of sensitivity to EBIs of barley powdery mildew populations (Fletcher and Wolfe, 1981; Wolfe et al., 1983). These observations once more confirm the validity of the advice to reduce the number of treatments as a strategy to prevent the development of resistance to fungicides.

In 1984, no decrease in sensitivity below the 1983 level was apparent. As isolates were only collected until July 1984, no definite conclusions on changes in sensitivity to EBIs in that year can be given.

The change in sensitivity to EBIs described in this report, has not been as sudden as the change in sensitivity to benzimidazoles (Kooistra et al., 1972) and to dimethirimol (Bent et al., 1971). Apparently, the population slowly adapts to EBIs.

The slow adaptation of *S. fuliginea* to EBIs is probably a case of directional selection. A rapid selection for higher resistance to EBIs in barley powdery mildew was considered unlikely, because resistance was not controlled by one major gene, but probably by a complex genetic system (Hollomon et al., 1984). Resistance to imazalil in *Aspergillus nidulans* was also determined by at least 10 different genes (Van Tuyl, 1977).

In England, Scotland and the Federal Republic of Germany a gradual selection for resistance to EBIs occurred over the years in wheat and barley powdery mildew (Fletcher and Wolfe, 1981; Wolfe et al., 1982, 1983, 1984; Bennett and Van Kints, 1983; Limpert and Fischbeck, 1983; Butters et al., 1984; Heaney et al., 1984). Decreased sensitivity of cereal powdery mildew to EBIs in the Federal Republic of Germany and the Netherlands was described by Buchenauer (1984) and De Waard et al. (1984). In cereals, the decreased sensitivity has not yet led to a complete loss of powdery mildew control by EBIs. However, certainly the efficacy of EBIs which inhibit C-14 demethylation (DMIs) is declining, and growers are inclined to use morpholines instead (Gilmour, 1984).

The fitness of the EBI-resistant isolates of *S. fuliginea* is hardly reduced (Schepers, 1985). The hypothesis of a gradual selection for higher resistance in spite of the breaks in the growing seasons (winter), during which no EBIs are used, is in line with these laboratory observations.

Erysiphe graminis f. sp. *hordei* isolates with a decreased sensitivity to EBIs also appeared to be as pathogenic as the wild-type isolates (Butters et al., 1984). Although periods with a low selection pressure by EBIs are longer in cereal crops than in cucumber crops, the sensitivity to EBIs of wheat and barley powdery mildew has now decreased for four years in succession, suggesting that the fitness of these isolates is normal under field conditions.

Positively correlated cross-resistance to DMIs was observed in various fungi (cf. De Waard and Fuchs, 1982). The cross-sensitivity of *S. fuliginea* isolates to bitertanol, buthiobate, fenarimol, imazalil and triforine is in agreement with these observations (Table 5; Schepers, 1983). Isolates with a decreased sensitivity to DMIs were as sensitive to fenpropimorph as the reference isolates. This lack of cross-resistance seems to be the rule for powdery mildews and might be usable to prevent the build-up of DMI-resistant populations (De Waard, 1984). Unfortunately, full dosages of fenpropimorph and the other morpholine EBIs, dodemorph and tridemorph, are phytotoxic to cucumber plants. This handicap has been avoided in a mixture of 20% tridemorph and 23.3% nitrothal-isopropyl. This mixture exerted good control of powdery mildew with a decreased sensitivity to DMIs (Jennrich and Hayler, 1984).

Generally speaking, the risk of development of resistance to EBIs is lower than for other site-specific fungicides. However, the cropping system, the environmental conditions and the inherent nature of the fungus are also important factors that influence the development of resistance in practice. The closed environment of the glasshouse and the intensive chemical control of *S. fuliginea* excessively favour the selection of isolates resistant to fungicides. A large genetic variability within the pathogen population may facilitate its relatively rapid adaptation. All these factors seem to enhance the initially low risk of development of resistance to EBIs.

To slow down the shift towards still higher resistance levels, the use of EBIs has to be restricted. The advice to restrict the use of EBIs in space and time will only be

followed by growers when they realize the severity of the situation and when good non-EBIs or mixtures with multi-site inhibitors are available. Unfortunately, growers are only willing to follow such an advice after personal experience with resistance problems. Good extension by governmental officers and fungicide manufacturers may change this attitude.

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Samenvatting

Veranderingen gedurende een driejarige periode in de gevoeligheid van Sphaerotheca fuliginea voor ergosterolbiosyntheseremmers in Nederland

Uit komkommerkassen in Nederland werden gedurende de periode 1981-1984 isolaten van *Sphaerotheca fuliginea* verzameld. De gevoeligheid van deze isolaten voor fenarimol en imazalil, twee ergosterolbiosyntheseremmers (EBR's), werd getest. De gegevens verzameld in 1981 wijzen erop dat de gevoeligheid voor EBR's lager was dan die van referentie-isolaten. In 1982 en 1983 daalde de gevoeligheid voor EBR's nog verder. In 1984 werden gegevens verzameld tot en met juli. Er werd geen significant verschil in gevoeligheid waargenomen met het niveau van 1983.

Isolaten verzameld in Limburg, waar EBR's minder vaak toegepast worden dan in Pijnacker, hadden een significant hogere gevoeligheid voor EBR's dan isolaten uit Pijnacker.

Naast verschillen in gevoeligheid voor EBR's tussen jaren, werden ook verschillen binnen de jaren waargenomen. In het begin van het groeiseizoen in 1983, voordat fungiciden waren gebruikt, was de gevoeligheid voor fenarimol en imazalil hoger dan later in het groeiseizoen.

In het algemeen hadden de veranderingen in gevoeligheid voor EBR's geen volledig falen van de bestrijding tot gevolg. In de meeste gevallen was een verkorting van de intervallen tussen de toepassingen van fenarimol en imazalil voldoende om te compenseren voor de verminderde gevoeligheid en om een goede bestrijding te verkrijgen. De resultaten benadrukken nogmaals de noodzaak om strategieën te ontwerpen die resistentie tegen EBR's voorkomen.

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