Neth. J. Pl. Path. 91 (1985) 65-76

Fitness of isolates of Sphaerotheca fuliginea resistant or sensitive to fungicides which inhibit ergosterol biosynthesis

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Accepted 6 December 1984

Abstract

Isolates of *Sphaerotheca fuliginea* resistant to fungicides which inhibit ergosterol biosynthesis (EBIs: bitertanol, fenarimol, imazalil) had been collected from glasshouses in the Netherlands. Fitness of these isolates was compared to that of isolates with a wild-type sensitivity to EBIs. Fitness parameters studied were germination of conidia, growth of germ tubes and mycelium, penetration, sporulation and competitive ability.

In an experiment in which 10 EBI-resistant isolates were compared to 7 wild-type isolates, one or more values of fitness parameters for EBI-resistant isolates were slightly lower than those for the wild-type isolates. However, within the group of resistant isolates no relation existed between the degree of resistance to EBIs and the degree of fitness. In an experiment with fewer isolates but with more replicates, differences in fitness between EBI-resistant and wild-type isolates were not detected over a three-month period.

In competition experiments in which no crowding was present, resistant isolates competed well with the wild-type isolate.

It is concluded that the hypothesis that resistance to EBIs is unlikely to develop under practical conditions because of decreased fitness of EBI-resistant strains, does not seem to hold for *S. fuliginea.*

Additional keywords: cucumber powdery mildew, bitertanol, fenarimol, imazalil.

Introduction

In the Netherlands cucumber powdery mildew *(Sphaerotheca fuliginea)* is primarily controlled by fungicides which inhibit ergosterol biosynthesis (EBIs: bitertanol, fenarimol, imazalil, triforine). The development of fungal resistance to EBIs under practical conditions was considered rather unlikely, a conclusion mainly based on the observation of a reduced fitness of isolates resistant to EBIs (Fuchs and Drandarevski, 1976). However, since an increasing number of isolates of several fungi with a decreased sensitivity to EBIs was observed in vitro (cf. Fuchs and De Waard, 1982) and in vivo (Wolfe et al., 1983; Butters et al., 1984; El-Goorani et al., 1984), *S. fuliginea* could be assumed to be able to gradually develop resistance to EBIs under practical conditions. Indeed, isolates of *S. fuliginea* were found with such a level of resistance to EBIs that control was impaired in some cases (Huggenberger et al., 1984).

The present study was undertaken to determine whether the fitness of isolates of *S. fuliginea* resistant to EBIs was comparable to that of isolates with a wild-type sen-

sitivity to these fungicides. Fitness is considered here to include all factors favourable to the production of progeny (Hartl, 1980).

Materials and methods

Plants. Cucumber plants *(Cucumis sativus* L.) cv. Lange Gele Tros were grown in a growth chamber with a light regime of 16 hours light (Philips TLMF 40W/35S, 7000 lux) at 18 °C and 80% relative humidity. Seeds were sown in 15-cm plastic pots containing steamed soil. Leaves of 30-day-old plants were used for the experiments.

Chemicals. Bitertanol (technically pure) was kindly provided by Bayer A.G., Leverkusen, Fed. Rep. Germany; fenarimol (technically pure) by Eli Lilly Research Centre Ltd., Surrey, England; imazalil (technically pure) by Janssen Pharmaceutica, Beerse, Belgium.

Toxicity assay. EC_{50} values of bitertanol, fenarimol and imazalil for inhibition of mildew development were determined according to Schepers (1984).

Fungal isolates. Isolates of *S. fuliginea* consisted of conidia from several diseased cucumber leaves, collected in a small area (1 to 2 m^2) in a glasshouse. At the time of the experiments the S isolates were sensitive and the R isolates were resistant to EBIs. Isolates S1, S2, S6 and S7 were maintained for more than five years without being exposed to fungicides at Eli Lilly (England), the Laboratory for Phytopathology (the Netherlands), Dr Maag A.G. (Switzerland) and Bayer A.G. (Fed. Rep. Germany), respectively; isolate S3 originated from a glasshouse in the USA where no EBIs had ever been used; isolates S4, S5, S8 and R11 were collected in Dutch cucumber glasshouses in October 1981 and were regularly transferred to fungicide-free plants during two years; isolates R1, R2, R5, R7, R9 and R10 were collected in Dutch cucumber glasshouses in October 1983; isolate R3 was isolated from a fenarimol-treated cucumber field in Israel in July 1982; isolate R4 was obtained from R3 by regular transfer to fenarimol-treated plants during one year; isolate R6 was collected in an imazalil-treated crop in England in September 1983; isolate R8 was collected in a triforine-treated crop in Norway in September 1983; isolate R12 was obtained from S8 by regular transfer to bupirimate-treated plants during one year. In this report no experiments are presented with isolate S8 itself. The isolates were maintained by subculturing on cucumber plants in the greenhouse (17-23 °C, 60-80% rh) every two weeks.

Determination of fitness parameters. Plants were inoculated by bringing them in contact with heavily infected leaves. Ten days later leaves, covered with profusely sporulating mycelium, were used for the experiments. Mildew-free leaves were detached and placed at the bottom of a 35-cm high PVC cylinder and inoculated by tapping a small portion of an infected leaf at the top of the cylinder. After inoculation, the complete leaves were floated on water in Petri dishes (ϕ 14 cm) and placed in a growth chamber (16 h light, 7000 lux, 20 °C, 60-80% rh). The Petri dishes were left half open to avoid excess humidity.

To determine the germination percentage of conidia (GPC), the number of ger-

mination tubes per conidium (GTC), the number of branches per hypha (BRH) and the number of penetration sites per conidium (PSC), leaf sections were excised from inoculated leaves after 21 hours (GPC) and after 46 hours (GTC, BRH, PSC). Sections were cleared by boiling in an acetic acid (99%)-methanol (96%) mixture (3:1) for 10 minutes. Then, the sections were rinsed in water, dehydrated in 1.0 N NaOH for at least 45 minutes, rinsed in water and mounted in 0.1% aniline blue in Sørensen's phosphate buffer (pH 6.6). Using a light microscope GPC was determined by observing 500 to 1000 conidia distributed over three leaves. Using fluorescence microscopy, penetration sites were easily detected by means of autofluorescence (Abul-Hayja, 1982; Kunoh et al., 1982). Together with PSC, GTC and BRH were determined using a Leitz Ploemopak fluorescence microscope with a BP 340-380 exciter filter and a LP 430 barrier filter.

Separately growing mildew colonies were selected on cucumber leaves at six days after inoculation. The number of conidiophores per colony (COC) were counted using a dissecting microscope.

The number of sporulating mildew colonies resulting from inoculation with dry conidia or with conidia in conidial suspensions were counted after incubation of cucumber leaves in Petri dishes at 20 °C for five to six days. Inoculation of dry conidia was performed by tapping a disc (ϕ 12 mm), which was totally covered with sporulating mycelium, at the top of the inoculation cylinder. Conidial suspensions, containing conidia from 25 heavily infected leaf discs, were incubated at room temperature for two days and applied to cucumber leaves with a De Vilbiss sprayer.

The competitive ability of *S. fuliginea* isolates was studied in two different types of experiments. In the type I experiment, plants were inoculated with a conidial suspension containing equal quantities of fungicide-resistant (R) and fungicide-sensitive (S) conidia. When, after 14 days, the leaves were completely covered with sporulating mycelium, the conidia were harvested and used for inoculation of a new batch of plants. After each transfer the ratio between the R and S conidia was assessed in the following way. Plants were inoculated with a diluted suspension of conidia, harvested from the test plants, which resulted in the appearance of separate colonies. From these leaves 100 discs (ϕ 12 mm), each with only one colony, were punched out and floated on a 3 μM carbendazim solution. Although isolates with a decreased sensitivity to EBIs had multiple resistance to benzimidazoles, carbendazim discriminated more accurately between EBI-resistant and EBI-sensitive conidia than any EBI. Therefore, resistance to benzimidazoles was used as a marker for EBI resistance. After incubation in a growth chamber (20 °C) for six days, the colonies which were inhibited (S) and which were not inhibited (R) in their development were counted.

In the type II experiment an epidemic was allowed to develop on plants in a greenhouse. The dispersal of conidia throughout the compartment was ensured by using a fan for 30 to 45 minutes each day.

Changes in the R:S ratio were determined periodically with the aid of trap plants. Trap plants were placed among the diseased plants for 15 minutes to six days, depending on the severity of the epidemic. Once colonies had developed, 100 discs with one colony each were floated on a 3 μM carbendazim solution.

Table 1. Toxicity of bitertanol, fenarimol and imazalil to growth of various *Sphaerotheca fuliginea* isolates in leaf disc tests in 1983.

Isolate	EC_{50} (μM)		
	bitertanol	fenarimol	imazalil
S1	0.17 (1.0)[1]	0.01 (1.0)	0.10 (1.0)
S2	0.21 (1.2)	0.01 (1.0)	0.15 (1.5)
S3	0.17 (1.0)	0.02 (2.0)	0.21 (2.1)
S4	0.30 (1.8)	0.02 (2.0)	0.21 (2.1)
S5	0.21 (1.2)	0.02 (2.0)	0.17 (1.7)
S6	0.20 (1.2)	0.01 (1.0)	0.15 (1.5)
S7	0.17 (1.0)	0.01 (1.0)	0.10 (1.0)
R1	1.70 (10)	0.45 (45)	4.50 (45)
R2	1.70 (10)	0.45 (45)	3.00 (30)
R3	1.00 (6.0)	0.55 (55)	3.00 (30)
R4	1.50 (9.0)	1.00 (100)	5.50 (55)
R5	1.00 (6.0)	0.30 (30)	1.70 (17)
R6	1.50 (9.0)	0.30 (30)	2.10 (21)
R7	1.70 (10)	0.45 (45)	4.50 (45)
R8	0.55 (3.2)	0.06 (6.0)	0.45 (4.5)
R9	1.70 (10)	0.55 (55)	5.50 (55)
R10	1.50 (9.0)	0.65 (65)	5.50 (55)

[1] Between brackets: resistance level defined approximately as ratio between EC_{50} of fungicide for each isolate and isolate S1.

Results

Toxicity of EBIs. EC_{50} values of bitertanol, fenarimol and imazalil for inhibition of *S. fuliginea* isolates in leaf disc tests are presented in Table 1. Isolates S1, S2, S3, S6 and S7, which had never been in contact with any fungicide, showed EC_{50} values of bitertanol of 0.17-0.21 μM, of fenarimol of 0.01-0.02 μM and of imazalil of 0.10-0.21 μM. The EC_{50} values of the EBIs for inhibition of isolates S4 and S5, which had regularly been transferred during two years to fungicide-free plants, fell in the same ranges.

All R isolates with the exception of R8, had EC_{50} values of EBIs as follows: bitertanol, 1.00-1.70 μM; fenarimol 0.30-1.00 μM; and imazalil, 1.70-5.50 μM. The degree of resistance was low for bitertanol, intermediate for imazalil and high for fenarimol. Isolate R4, obtained after regular transfer to fenarimol-treated plants during one year, could tolerate 100 times as much fenarimol as isolate S1. A positively correlated cross-resistance to bitertanol, fenarimol and imazalil was observed in all isolates.

To check the stability of resistance to EBIs, the toxicity of fenarimol to growth of some isolates was determined during three years (Table 2). Isolates S4 and S5, collected in 1981, originally possessed a decreased sensitivity to fenarimol, but had lost this property by the end of 1983. The high sensitivity of isolate S7 and the intermediate sen-

Table 2. Toxicity of fenarimol to growth of various *Sphaerotheca fuliginea* isolates in leaf disc tests in 1982, 1983 and 1984.

Isolate	$EC_{50}(\mu M)$ fenarimol		
	1982	1983	1984
S4	0.45	0.20, 0.30, 0.02	0.01
S5	0.17, 0.30[1]	0.30, 0.02	0.01, 0.01
S7	0.01, 0.01	0.01, 0.01	0.01, 0.01
R3	–	0.55, 0.55	0.55, 1.00
R9	–	0.55	0.55
R10	–	0.45	0.45
R11	0.08, 0.10	0.21, 0.10	0.10

[1] More EC_{50} values per isolate represent the sensitivity to fenarimol in different parts of the years.

Table 3. Germination of conidia and formation of hyphae, penetration sites and conidiophores of isolates of *Sphaerotheca fuliginea* resistant or sensitive to fungicides which inhibit ergosterol biosynthesis.

Isolate	Fitness parameters[1,2,3]				
	GPC	GTC	BRH	PSC	COC
S1	45	3.1	1.0	4.4	58
S2	49	3.0	0.8	4.8	51
S3	48	3.2	0.9	4.0	55
S4	49	3.1	1.2	3.6*	39
S5	40	2.7*	1.0	3.7	53
S6	38*	3.2	1.0	4.4	38
S7	45	3.1	0.7	3.7	38
R1	31*	3.1	1.1	4.5	52
R2	39*	2.7*	0.6*	3.4*	53
R3	45	2.4*	0.7	3.4*	45
R4	40	3.2	0.9	3.4*	33*
R5	39*	2.5*	0.8	4.3	26*
R6	31*	3.1	0.7*	3.8*	39
R7	35*	2.8	0.7	3.5*	26*
R8	35*	2.8	0.6*	3.9	34*
R9	30*	2.6*	0.5*	2.5*	51
R10	27*	3.0	0.7	3.6*	22*

[1] GPC: germination percentage of conidia; GTC: germination tubes per conidium; BRH: branches per hypha; PSC: penetration sites per conidium; COC: conidiophores per colony.
[2] Asterisk indicates values significantly different from those of S1 (χ^2 test, $p = 0.05$).
[3] Figures indicate average values of 500 to 1000 (GPC), 50 (GTC, BRH, PSC) and 30 (COC) observations, obtained after one inoculation.

sitivity of R11 to fenarimol did not change during the years of investigation. The isolates R3, R9 and R10 with a low sensitivity to fenarimol, did not revert to the wild-type sensitivity during one year of subculturing on fungicide-free plants.

Fitness parameters. Determination of a number of fitness parameters (GPC, GTC, BRH, PSC, COC) after one inoculation experiment, showed that three S isolates, viz. S4, S5 and S6, possessed fitness parameters which were lower than those of isolate S1. All R isolates showed one or more values of fitness parameters which were lower than those of S1. In comparison to S1, the values of the fitness parameters of the R isolates varied in the following way: GPC 60-100%, GTC 77-103%, BRH 50-110%, PSC 57-102% and COC 38-91% (Table 3). Per R isolate, one to four but never all five fitness parameters were lower than those of S1.

Within the group of R isolates no correlation seemed to exist between the degree of resistance to EBIs and the values of the fitness parameters observed. Isolate R4, the isolate with the highest degree of resistance to fenarimol, did not show lower fitness parameters than isolate R8, an isolate with a low level of resistance to EBIs.

Results from five different inoculation experiments did not show significant differences between the values of the fitness parameters of the isolates S1, S2, R3 and R10 (Table 4).

The frequency distribution of the numbers of sporulating colonies resulting from inoculation with dry conidia of isolates S1 and S2 was similar to that of isolates R3 and R10 (Fig. 1). The variation in colonies per leaf was substantial, but was similar for the R and S isolates. This result contrasted with the result obtained after inoculation with conidial suspensions, when the isolates S1 and S2 produced significantly fewer colonies per leaf than the isolates R3 and R10.

The ability of several isolates to compete with isolate S6 was tested in two types of competition experiments (Table 5). In the type I experiment, the S2 conidia disappeared gradually from the population. The percentage of R11 conidia varied considerably and this isolate eventually even appeared to represent a greater part of the

Table 4. Fitness parameters of *Sphaerotheca fuliginea* resistant or sensitive to fungicides which inhibit ergosterol biosynthesis.

Isolate	Fitness parameters[1,2,3]			
	GTC	BRH	PSC	COC
S1	3.3	1.3	4.3	33
S2	3.4	1.2	4.4	35
R3	3.5	1.1	4.3	30
R10	3.5	1.3	4.5	31

[1] GTC: germination tubes per conidium; BRH: branches per hypha; PSC: penetration sites per conidium; COC: conidiophores per colony.

[2] No significant differences were found (χ^2 test, $p = 0.05$).

[3] Figures indicate average values of 70 (GTC, BRH, PSC) and 110 (COC) observations distributed over five inoculation experiments.

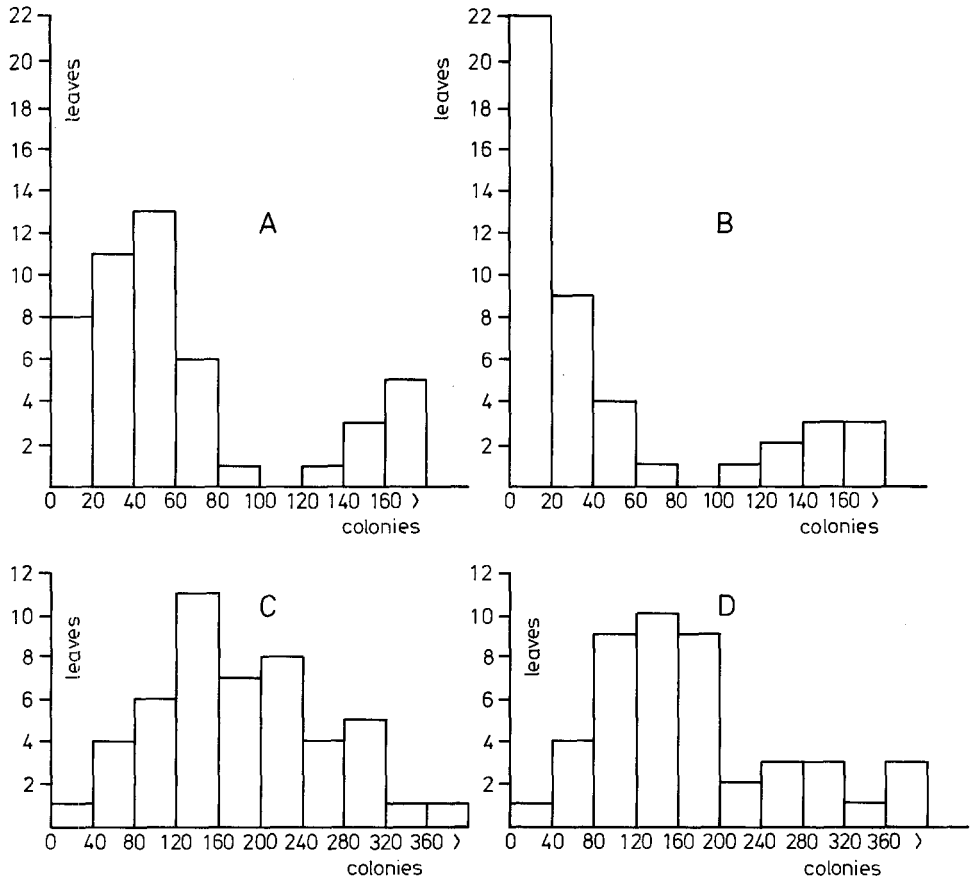

Fig. 1. Frequency distributions of the number of sporulating colonies resulting from inoculation with dry conidia and with conidial suspensions of *Sphaerotheca fuliginea* isolates. Isolates were resistant (R3, R10) or sensitive (S1, S2) to fungicides which inhibit ergosterol biosynthesis.
1) A: conidial suspensions of R3 and R10, B: conidial suspensions of S1 and S2, C: dry conidia of R3 and R10, D: dry conidia of S1 and S2.
2) Data were obtained from 48 leaves distributed over five inoculation experiments.
3) A was significantly different from B; C was not significantly different from D (median test, $p = 0.05$).

population than isolate S6. A contradiction in results was observed between the two type I experiments in which isolate S5 was involved. In the type II experiment, the S4:S6 and R12:S6 ratios did not change in the course of the experiment.

Discussion

Fitness is a comparative concept, representing the reproductive success of an individual or a population in comparison to that of other individuals or populations.

Table 5. Competition between *Sphaerotheca fuliginea* isolates resistant to fungicides and the reference isolate S6 on cucumber plants in the absence of any fungicide.

Weeks after inoculation	Proportion of resistant isolate (%)[1]					
	type I experiment[2]				type II experiment[3,4]	
	S2	S5a	S5b	R11	S4	R12
0	53	49	59	70	62	72
2	40	33	62	60	62	83
3	–	–	–	–	69	78
4	30	13	41	49	–	83
5	–	–	–	–	65	79
6	50	19	41	37	64	–
7	–	–	–	–	53	–
8	25	16	26	92	–	–
10	15	80	9	99	–	–

[1] Isolates S2, S5, R11, S4 and R12 had EC_{50} values of fenarimol of 1, 30, 8, 20 and 20 μM, respectively. Isolate S5 was tested twice, viz. as S5a and S5b.
[2] Regular transfer of conidia to mildew-free plants by means of conidial suspensions.
[3] An epidemic was allowed to develop on a set of plants.
[4] Relative rate of disappearance (r_d): regression coefficient of the logit line of the R isolate (Zadoks, 1982). The r_d values for the isolates S4 and R12 differed hardly from zero.

It does not depend on one gene but on the entire genotype (Hartl, 1980). This implies the risk that not all observed differences in fitness can be attributed to the differential sensitivity to a fungicide. The best way to eliminate side-effects on fitness is to work with two isolates which are genetically identical and which only differ from each other in the sensitivity to the fungicide. Such an approach depends on the development of resistance under controlled conditions. This has not yet been achieved with *S. fuliginea*. An indication of fitness has therefore to be obtained by comparing many isolates collected from commercially treated crops to many reference isolates with a wild-type sensitivity to EBIs.

Stability of resistance. To find out whether the decreased sensitivity to EBIs was a temporary adaptation or a stable, genetically determined, characteristic, isolates were transferred to fungicide-free plants every two weeks. None of the isolates lost its resistance within a period covering 25 to 30 transfers. This confirms the findings of others, who also reported a stable resistance to EBIs (Fuchs et al., 1977; Walmsley-Woodward et al., 1979; De Waard et al., 1982; Huggenberger et al., 1984).

Two isolates (S4 and S5) reverted to the wild-type sensitivity within a period of two years during which no selection pressure was present. Genetic heterogeneity of the isolates, mutation, and contamination with wild-type conidia might have caused this gradual shift towards the wild-type sensitivity.

Fitness parameters. In several fungi, the values of fitness parameters of EBI-resistant

strains selected in vitro were lower than those of the strains from which they had been derived (Fuchs and Viets-Verweij, 1975; Fuchs et al., 1977; Buchenauer, 1977, 1983; De Waard and Gieskes, 1977). In some cases an unchanged fitness was found (De Waard et al., 1982).

It seems unlikely that in laboratory experiments all strains can be detected that will arise under practical conditions. First, the short duration of the selection pressure in vitro prevents a selection for higher fitness. Second, the limited size of the population in vitro will prevent the detection of rare mutants with a high fitness. Therefore, pathogen isolates from crops treated for a number of years with EBIs will give a better indication of a possible correlation between EBI resistance and fitness. Barley and wheat powdery mildew isolates collected from such crops sometimes showed a decreased fitness (Walmsley-Woodward et al., 1979; Buchenauer, 1984) and sometimes a normal fitness (Laws et al., 1982; Butters et al., 1984).

Different fitness parameters can be studied, which vary in their effect on reproductive success. The ideal situation is reached when the fitness of an isolate is accounted for by all parameters together. Such data can be collected by observing individual conidia throughout the infection process. Since no techniques were available to follow the infection process through time, several steps in the infection process were studied separately.

When the fitness parameters of 7 S and 10 R isolates were determined, using data from one inoculation experiment, the group of R isolates appeared to have a slightly lower fitness than the group of S isolates (Table 3). However, several values of parameters of individual isolates were not lower than those of the S isolates. Moreover, the fitness parameters of selected isolates, two S and two R isolates, obtained from five inoculation experiments, did not reveal any effect of fungicide sensitivity on fitness (Table 4). The replication in time might have obscured the small differences observed in the case of one inoculation experiment.

The absence of a relation in the group of R isolates between degree of resistance and fitness might indicate that the observed small differences were caused by a different genetic background and not by EBI resistance. In other cases, in which isolates with a similar background were used, a negative relation was found between EBI resistance and fitness (Fuchs et al., 1977; De Waard and Gieskes, 1977).

The similarity in the frequency distributions of the number of colonies resulting from inoculation with dry conidia of two R and two S isolates indicates that any slight differences in fitness parameters do no necessarily result in a lower infection. Compensation, a phenomenon described earlier for *S. fuliginea,* might play a role here (Bashi and Aust, 1980).

Epidemiologically irrelevant but interesting was the observation that inoculation with conidial suspensions of R isolates resulted in more mildew colonies than with conidial suspensions of S isolates. This phenomenon might be related with the mechanism of resistance to EBIs and needs further investigation.

Competition. Experiments in which EBI-resistant and EBI-sensitive isolates are inoculated on the same set of plants, will provide information on the competitive ability of these isolates. The set-up of the experiment, in which a crowded (type I) or an uncrowded (type II) situation is created, will greatly influence the fitness parameters that contribute to the competitive ability of the isolates.

Competition experiments reported in the literature all placed the R and S isolates in a crowded situation. The density of conidia administered was so high that only some of them could develop (Hollomon, 1978; Dekker and Gielink, 1979; De Waard et al., 1982; Zadoks, 1982). This type of competition experiment corresponds with the type I experiment. Two R isolates gradually disappeared from the population, but two others competed well with the wild-type isolate S6. A reduction in the number of R conidia in a similar experiment was observed in *S. fuliginea* isolates resistant to benzimidazoles (Zadoks, 1982) and pyrazophos (Dekker and Gielink, 1979). It can be attributed to a weaker competitive ability of the R isolates under such circumstances. However, a differential sensitivity of R and S conidia to water might affect the competitive ability in such a way that the R isolates increase, as was observed in the isolates S5a and R11. That not all factors involved in this type of competiton experiment are understood, is indicated in the contradictory results obtained with the S5 isolate.

Since crowded situations do not normally occur in commercial crops, competition experiments of type II, in which no crowding with competition for space or nutrients is present, will approach the competitive ability under practical conditions better than the type I experiment. The results of the type II experiment can provide r_d values. Such values, representing the relative rate of disappearance of the R isolates, should not be calculated from competition experiments with crowding, but only from data obtained in the early phase of an epidemic (Zadoks, 1982). The isolates S4 and R12 did not disappear from the population in the type II experiment. The r_d values differed hardly from zero, indicating that under the conditions of the experiment the competitive ability of these isolates was not lower than of the wild-type isolate S6.

Conclusions. Mainly on the basis of observations on fitness parameters of in vitro selected EBI-resistant strains, predictions were made about the development of resistance to EBIs under practical conditions (Fuchs and Drandarevski, 1976; Fuchs et al., 1977; De Waard and Gieskes, 1977; Dekker, 1982). The original observations of decreased fitness in combination with low levels of resistance led to the hypothesis that resistance to EBIs was unlikely to develop under practical conditions. Observations on strains resistant to dicarboximide fungicides led to similar predictions (Beever and Byrde, 1982).

However, a prolonged and exclusive use of dicarboximide fungicides finally resulted in cases of failure of control (Leroux and Besselat, 1984). Since EBI-resistant isolates of *Penicillium italicum* were found with a normal fitness, the risk of development of resistance to EBIs is considered real (De Waard et al., 1982). The finding of *P. italicum* isolates with a decreased sensitivity to EBIs in packinghouses in Egypt by El-Goorani et al. (1984) confirms the seriousness of the warning of De Waard et al. (1982).

The fitness of EBI-resistant *S. fuliginea* isolates, collected in commercial glasshouses, was hardly reduced. Under practical conditions resistance to EBIs can develop gradually, provided that a pathogen prone to develop resistance to fungicides is subject to a prolonged selection pressure of EBIs (Schepers, 1983; Huggenberger et al., 1984). As EBIs are still being used almost exclusively to control cucumber powdery mildew in the Netherlands, continued selection for higher levels of resistance and fitness does not seem unlikely.

Acknowledgements

The author wants to thank Anton Franken, Kees Hertogh, Peter van de Homberg, Piet van Marrewijk, Lynn Moore and Stan Roelofs for their assistance. Thanks are also due to Dr J. Dekker, Ir H.D. Frinking, Dr A. Fuchs, Dr M.A. de Waard and Dr J.C. Zadoks for their advice and support, and for critically reading the manuscript.

Samenvatting

'Fitness' van isolaten van Sphaerotheca fuliginea die resistent of gevoelig zijn voor fungiciden die de ergosterol-biosynthese remmen

Uit kassen in Nederland waren isolaten van *Sphaerotheca fuliginea* verzameld, die resistent waren tegen fungiciden die de ergosterol-biosynthese remmen (EBR's: bitertanol, fenarimol, imazalil). De 'fitness' van deze isolaten werd vergeleken met die van isolaten met een wild-type gevoeligheid voor EBR's. De volgende 'fitness'-parameters werden bestudeerd: sporekieming, groei van kiembuizen en mycelium, penetratie, sporulatie en competitievermogen.

In een proef, waarin 10 EBR-resistente isolaten werden vergeleken met 7 wild-type isolaten, waren één of meer 'fitness'-parameters iets lager dan die van de wild-type isolaten. Binnen de groep van de resistente isolaten bestond geen relatie tussen de mate van resistentie tegen EBR's en de waarden van de 'fitness'-parameters. In een proef met iets minder isolaten maar met meer herhalingen in de tijd, werden geen verschillen in 'fitness' waargenomen tussen de EBR-resistente en wild-type isolaten. In competitieproeven waarin een epidemie zich kon ontwikkelen, concurreerden de resistente isolaten goed met het wild-type isolaat.

Er wordt geconcludeerd dat de hypothese dat resistentie tegen EBR's in de praktijk waarschijnlijk niet zou optreden vanwege een verminderde 'fitness' van de EBR-resistente isolaten, niet van toepassing is op *S. fuliginea.*

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