Development and persistence of resistance to fungicides in *Sphaerotheca fuliginea* in cucumbers in the Netherlands

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Proefschrift
ter verkrijging van de graad van
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Stellingen

I

Resistance to fungicides in _Sphaerotheca_ _fuliginea_ is a (cu)cumbersome problem.

Dit proefschrift.

II

Het gegeven dat resistentie van _Sphaerotheca_ _fuliginea_ tegen fungiciden die de ergosterolbiosynthese remmen bepaald wordt door meer dan één gen impliceert de aanwezigheid van meer dan één resistentiemechanisme.

Hollomon, D.W., Butters, J. en Clarck, J., 1984. Br. Crop Prot. Conf. (1984): 477-482.

III

Het gebruik van de term fungicide-resistentie door boer en tuinder wordt niet zo zeer bepaald door de mate van ongevoeligheid van een schimmel voor een fungicide, als wel door de economische implicaties van die ongevoeligheid.

H.T.A.M. Schepers, 1985. Neth. J. Pl. Path. 91: 105-118.

IV

Het verdient aanbeveling op etiketten van bestrijdingsmiddelen die gebruikt worden in de glastuinbouw in de gebruiksaanwijzing betere richtlijnen betreffende de toegestane hoeveelheid bestrijdingsmiddel per oppervlakte-eenheid op te nemen.

V

Zij die één jaar vooruit kijken ontwikkelen strategieën om de ontwikkeling van resistentie tegen fungiciden te vertragen. Zij die tien jaar vooruit kijken maken bestrijdingsmiddelen met een laag risico op ontwikkeling van resistentie. Zij die honderd jaar vooruit denken ontwikkelen de mens zelf.

Naar een Chinees gezegde.

VI

Er is een grote kans dat binnen afzienbare tijd in de tarweteelt een verminderde gevoeligheid van tarwemeeldauw voor fenpropimorf zal optreden.

VII

Het is onwaarschijnlijk dat multinationals een planteveredelingsprogramma koppelen aan de ontwikkeling van landbouwchemicaliën.

Mooney, P.R., 1979. Seeds of the earth. Mutual Press Limited, Ottawa.

VIII

Fabrikanten van gewasbeschermingsmiddelen beweren dat deze erg belangrijk zijn voor de produktie van meer en beter voedsel voor een hongerige wereld; zij onderschatten de mogelijkheden van, en de behoefte aan, een niet-chemische oplossing van de verbetering van de voedselproduktie.

IX

Ook bij biologische bestrijding van plagen is ontwikkeling van resistentie mogelijk: enige bescheidenheid bij het propageren van biologische bestrijding zou derhalve gepast zijn.

Muldrew, J.A., Can.J. Zool.
31: 313-332.

X

De van oudsher scherp omschreven grens tussen potplantenteelt, vasteplantenteelt en boomteelt zal door de tendens tot assortimentsverbreding steeds verder vervagen.

XI

De opkomst bij jaarvergaderingen van federaties dient niet verhoogd te worden door boetes te heffen bij afwezigheid, maar door de agenda voor de clubs relevant te maken.

Huishoudelijk reglement KNAU,
Artikel 113.

XII

Eenheid in de richting waarin rugtitels van boeken worden gedrukt zou de nekspieren sparen.

XIII

Strips tease.

H.T.A.M. Schepers,

Development and persistence of resistance to fungicides in Sphaerotheca fuliginea in cucumbers in the Netherlands.

Wageningen, 25 september 1985.

WOORD VOORAF

Graag wil ik allen bedanken die het verschijnen van dit proefschrift mede mogelijk hebben gemaakt.

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Een negental studenten heeft aan dit onderzoek meegewerkt. Hierbij wil ik Peter van den Homberg, Piet van Marrewijk, Heleen Bestevaar, Stan Roelofs, Lynn Moore, Anton Franken, Marion van Rosevelt, Kees Hertogh en Piet Spoorenberg bedanken voor hun werk en ideeën.

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CONTENTS

INTRODUCTION

Fungicides play an important role in the control of fungal plant diseases. Up to the 1960s, chemical control of plant diseases occurred with protectant fungicides. Then, systemic fungicides became available and they gradually conquered part of the fungicide market. Systemic fungicides are taken up by the plant and translocated within the plant tissue. Over protectant fungicides, systemic fungicides have the advantage to eliminate a pathogen after it has penetrated into the plant. It is evident that they have to be selective in their action; they should inhibit the fungus without adversely interfering with the plant's metabolism Although protectant fungicides still dominate the scene in terms of world consumption, they have been completely replaced by systemic fungicides in some crops. In these crops systemic fungicides have enabled growers to cultivate a crop all year round. Although the costs of systemic fungicides are only a small part (± 2%) of the total production costs, they are considered an insurance fee for the growers' profit. However, this insurance is not always as reliable as initially hoped for. In some cases the frequent use of systemic fungicides selects fungicide-resistant strains which render the fungicide less effective or in the worst case completely ineffective. Resistance to fungicides is now an important field of research (Dekker and Georgopoulos, 1982).

The build-up of resistance in the field depends on many factors, among which the type of fungicide used and the nature of the disease. With some fungicides, for example the benzimidazoles and dimethirimol, development of resistance happened so fast that problems arose already shortly after their introduction. With other fungicides no disease control problems arose, at least not during the first years. To the latter category belong the fungicides which inhibit ergosterol biosynthesis (EBIs). However, resistance in vitro was easily obtained. Considering the low level of resistance in vitro and the decreased fitness of resistant fungal strains, it was even once considered unlikely that resistance to EBIs would develop in practice (Fuchs and Drandarevski, 1976). It might be for these reasons, that EBIs became increasingly important in controlling plant pathogens. Especially in the control of powdery mildews EBIs are nowadays practically indispensable.

In spite of the fact that the risks for a development of resistance in practice were considered low, it could not be concluded that a long term use might eventually lead to development of resistance, as has been experienced in the case of *Botrytis cinerea* resistant to dicarboximides (Leroux and Besselat, 1984).

In view of this a study was undertaken to examine the sensitivity to EBIs of a fungal population intensively treated with EBIs for several years. In this way insight could be gained about development and persistence of resistance to EBIs and dispersal of EBI-resistant strains under practical conditions.

The organism chosen was the causal agent of cucumber powdery mildew, *Sphaerotheca fuliginea*. This pathogen caused only minor problems before 1965; the crop became infected several months after the start of the growing season. Protectant fungicides effectively controlled the disease (Besemer, 1965). Nowadays, cucumbers are cultivated at a much larger scale (700 ha) and almost all year round. These circumstances facilitated the occurrence of *S. fuliginea* and protectant fungicides could no longer control the disease satisfactorily. A frequent application of systemic fungicides became necessary. Several protectant and systemic fungicides were licensed for use in the Netherlands as mentioned in Table 1. After development of resistance to dimethirimol, benzimidazoles and pyrazophos, the growers now use primarily EBIs: bitertanol, fenarimol, imazalil and triforine.

Many factors favouring the development of resistance to fungicides, as mentioned by Dekker (1982), are inherent in the chemical control of *S. fuliginea* with EBIs. EBIs are applied frequently (1) as a foliar spray (2) and the application is thorough (3). The extent of the area treated is large (4), growers using EBIs almost exclusively (5). The pathogen sporulates on the aerial parts of the cucumber plant and develops quickly (6). The closed environment of the glasshouse prevents non-treated conidia to mix with the EBI-treated pathogen population (7). Together, these factors create a situation in which resistance to fungicides can develop readily, as was demonstrated with dimethirimol and benzimidazoles. When development of resistance to EBIs under practical conditions would be possible, there was a fair chance that it would be noticed in this particular crop-pathogen situation.

This thesis contains five papers. In the first paper the appearance of cucumber powdery mildew in the Netherlands and the dispersal between glasshouses is described. The second paper deals with the persistence of resistance to dimethirimol, benzimidazoles and pyrazophos, fungicides to which *S. fuliginea* developed resistance years ago. The presence of strains resistant to these fungicides, provides information on their long-term fitness. The short-term fitness of EBI-resistant strains was thought to be low, thus preventing the build-up of a resistant pathogen population in practice. This aspect was studied in the third paper. The sensitivity of *S. fuliginea* in cucumber glasshouses in the Netherlands from 1981 to 1984 is dealt with in the fourth and fifth paper.

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Dekker J. & Georgopoulos, S.G., 1982. Fungicide resistance in crop protection. Pudoc, Wageningen, pp. 265.

Fuchs, A. & Drandarevski, C.A., 1976. The likelihood of development of resistance to systemic fungicides which inhibit ergosterol biosynthesis. Neth. J. Pl. Path. 82: 85-87.

Leroux, P. & Besselat, B., 1984. Pourriture grise: La résistance aux fongicides de *Botrytis cinerea*. Phytoma 359: 25-31.

Table 1. Fungicides registered for control of cucumber powdery mildew in the Netherlands

Common name	Trade name	Type[1]	Year of introduction[2]	Recommended dosage[3]	Formulation[4]
benomyl	Benlate	S	1972	250	50% WP
bitertanol	Baycor	S	1983	300	30% EC
bupirimate	Nimrod	S	1982	500	25% EC
carbendazim	Bavistin	S	1972	250	50% EC
dimethirimol	Milcurb	S	1968	125	12.5% EC
dinocap	Karathane	P	1946	225	22.5% WP
fenarimol	Rubigan	S	1981	24	12% EC
imazalil	Fungaflor	S	1977	50	20% EC
pyrazophos	Curamil	S	1967	120	30% EC
tolylfluanid	Eupareen M	P	1978	1250	50% WP
triforine	Funginex	S	1972	200	20% EC

[1] S: systemic; P: protectant.

[2] Year of introduction in the Netherlands.

[3] Dosage in mg a.i. l^{-1}.

[4] WP: wettable powder, EC: emulsifiable concentrate.

A pattern in the appearance of cucumber powdery mildew in Dutch glasshouses

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Abstract

An inquiry was held in 1982, 1983 and 1984 to collect information on the survival and dispersal of cucumber powdery mildew. Growers who planted a crop in December, January or February were asked when they observed mildew for the first time in their crop.

A gradual increase in the number of infected crops was observed from planting until May. The earliest observations of infected crops were immediately after planting. The weeks of the first observation of mildew, the disease-free periods and the apparent rates of increase of infected crops are presented for various districts. In the districts with the highest crop density (Pijnacker), mildew was generally observed early in the growing season, while the apparent rate of increase of infected crops was higher than in other districts. In the district with the lowest crop density (Northern Netherlands), mildew was observed late in the growing season and the apparent rate of increase of infected crops was low. Possible means of survival and dispersal of inoculum are discussed. It is suggested that overwintering of inoculum is possible because cucumber plants are grown all year round. Dispersal of cucumber powdery mildew is suggested to take place by transportation of infected planting stock, visitors and wind.

Additional keywords: Cucumis sativus, Sphaerotheca fuliginea, dispersal, survival.

Introduction

Cucumber powdery mildew is a serious disease in cucumber crops; it reduces the assimilating leaf area. The two most recorded species are *Erysiphe cichoracearum* DC. emend. Salm. and *Sphaerotheca fuliginea* (Schlecht.: Fr.) Poll. As perithecia are rare, the conidial characteristics are used to identify these two species (Boesewinkel, 1980). In the Netherlands, cucumber powdery mildew was identified as *S. fuliginea* in 1964 (Boerema and Van Kesteren, 1964), and this has been the only species since then. Cucumber powdery mildew is generally favoured by dry conditions, moderate temperatures and reduced light intensity, but it can develop under a wide range of climatic conditions (Sitterly, 1978). In the glasshouse, powdery mildew conidia are dispersed by air currents (Frinking and Scholte, 1983). On the sources of the primary inoculum and the exchange of inoculum between glasshouses little information is available.

Glasshouse-grown cucumbers are, after tomatoes, the second vegetable crop in Dutch horticulture. The total area in 1983 was 733 ha, with an export value of 312 million guilders (Centraal Bureau voor de Statistiek, Voorburg, 1983). The cucumber cultivars, grown in soil or on artificial substrates, are 100% female, parthenocarpic and mildew-sensitive. The early production crops are planted in December, while the late crops are removed in November. Some growers have one crop from December/January until October. Others remove the crop in July/August and immediately plant a new one, which produces until November. Most growers buy 5-to-6-week-old cucumber plants from specialized nurseries. In order to provide the growers with plants in December, the nurseries have to sow cucumbers in October/November. Six to seven weeks after planting in the production glasshouses, the first cucumbers are harvested. In this way cucumbers are supplied throughout the year, except in December and January. Cucumber plants are continuously present, although on a variable area (Fig. 1).

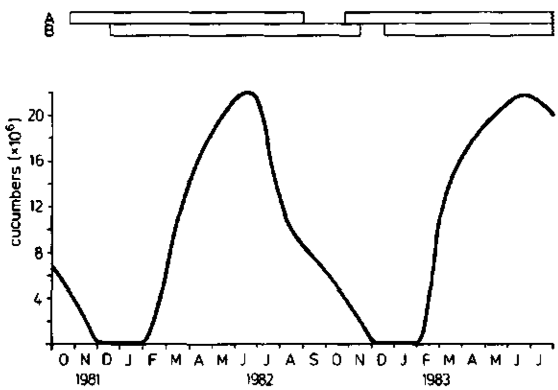

Fig. 1. Number of cucumbers (× 10^6) supplied at thirteen auction markets and presence of cucumber plants in nurseries (A) and production glasshouses (B) throughout the year.

An inquiry was held in 1982, 1983 and 1984 to collect information on the survival and dispersal of cucumber powdery mildew.

Materials and methods

Telephone inquiry. Information was obtained by telephone from growers who plant their cucumber crops in December, January or February. Crops planted in March, April or May were not included in the inquiry. Addresses were kindly provided by the extension service, the auction market of Pijnacker and the chemical industries. All available addresses were used, except from Pijnacker where a random selection was made (Fig. 2).

The inquiry was held in May 1982, 1983 and 1984 (week numbers 19, 20 and 21). Information was asked about the week of cucumber planting and the week of first observation of cucumber powdery mildew. To prevent negative week numbers, the first week in January was given number 5 (in 1982 and 1983) or 6 (in 1984). When at the time of the inquiry the cucumber crop was mildew-free, the hypothetical week number 35 was used for statistical purposes. Week number 35 was chosen because experience learned that by that time all growers had observed cucumber powdery mildew.

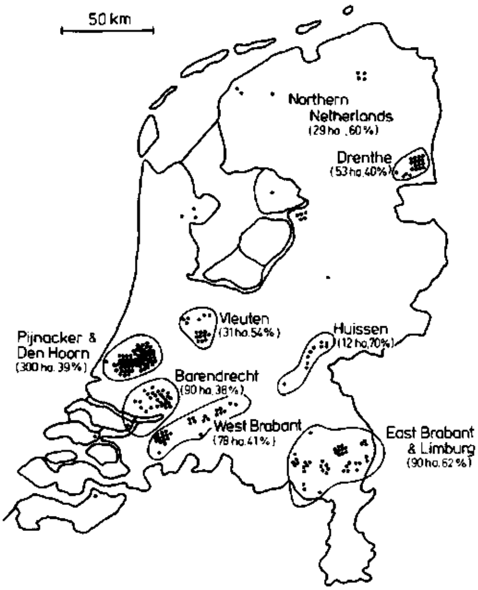

Fig. 2. Geographical distribution in the Netherlands of cucumber glasshouses selected for the inquiry. Tthe data between brackets refer to (1): total area including crops planted in December, January and February (78%) and crops planted in March, April and May (22%), and (2): estimated area selected for the inquiry as a percentage of the area planted in December, January and February (Centraal Bureau voor de Statistiek, 1983).

In this figure Pijnacker and Den Hoorn are presented as one district because no data were available on the area of cucumber crops in the separate districts.

Statistics. A student t-test was used to detect differences between the mean week numbers of the first observation of mildew (FOM, Table 1) and the means of disease-free periods (DFP, Table 2) for each district (Beyer, 1976). Differences in the distribution of FOM and DFP values were compared between 1982, 1983 and 1984, using the Kolgomorov-Smirnov test. The apparent rates of increase of infected crops (AIC, Table 3) were determined as described by Van der Plank (1963).

Results

Week of first observation of mildew. (FOM, Table 1, Figs 3 and 4).

1982. The earliest observation of mildew was in December, 1981. In the districts of Pijnacker and Den Hoorn the mean values of FOM were both 16 (medio March), which was earlier than in the other districts. In the districts of Huissen and Northern Netherlands the mean values of FOM were 32 and 28, respectively, which was later than in the other districts.

1983. The earliest observation of mildew was in January, 1983. In most districts the mean values of FOM were between 21 and 26 (April, May). In the districts of Drenthe and Northern Netherlands the mean values of FOM were 31, which was later than in the other districts.

1984. The earliest observation of mildew was in week 6 (January). In the district of Pijnacker the mean value of FOM was lowest, viz. 21 (medio April). In the other districts the mean values of FOM varied from 25 to 32.

Comparison 1982, 1983, 1984. In 1983 the mean values of FOM in the districts of Pijnacker and Den Hoorn were 5 and 8 weeks higher than in 1982. In 1984 FOM values in most districts were similar to those in 1983, except for the districts of Vleuten and East Brabant and Limburg, where mildew was observed later. In 1984 mildew was observed later than in 1982 in six districts. Only in the districts of Huissen, Drenthe and Northern Netherlands FOM values of 1984 were not higher than in 1982.

Table 1. Mean week numbers[1] of cucumber planting and of first observation of mildew in various districts in the Netherlands in 1982, 1983 and 1984.

District	Number of crops			Cucumber planting			First observation of mildew (FOM)		
	1982	1983	1984	1982	1983	1984	1982	1983	1984
Pijnacker	35	61	73	5	6	6	16 ab[2]	21 a x[3]	21 a x
Den Hoorn	37	31	28	5	6	6	16 a	24 ab x	26 bc x
Barendrecht	30	27	27	6	6	6	20 bc	24 ab	28 bcd x
Huissen	10	7	7	9	11	10	32 f	24 abcd	32 cd
Vleuten	15	13	12	7	7	7	22 cd	25 ab	30 bcd xxx
East Brabant and Limburg	46	43	41	8	7	7	24 de	23 ab x	26 b xx
West Brabant	28	25	25	8	7	7	22 cd	26 b d	29 bcd x
Drenthe	18	17	17	7	7	6	24 cde	31 c	25 ab
Northern Netherlands	17	14	12	9	9	9	28 ef	31 cd	32 d
Total	236	238	242	7	7	7	23	25 x	28 xxx

[1] First week of January is given number 5 (1982, 1983) or 6 (1984).
[2] Values in columns followed by the same letter are not significantly different (Student test, $p = 0.05$).
[3] x: distribution of values is significantly different from that in 1982, xx: distribution of values is significantly different from that in 1983, xxx: distribution of values is significantly different from those in both 1982 and 1983 (Kolgomorov-Smirnov test, $p = 0.05$).

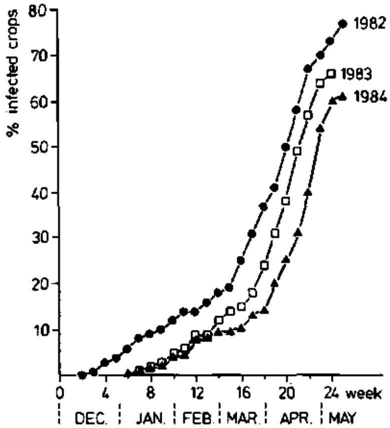

Fig. 3. Disease progress of cucumber powdery mildew in 1982, 1983 and 1984 in cucumber crops in the Netherlands.

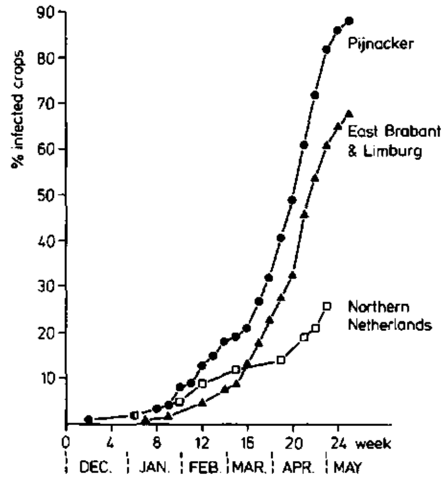

Fig. 4. Disease progress curves of cucumber powdery mildew in the districts of Pijnacker, East Brabant & Limburg and Northern Netherlands in 1982, 1983 and 1984. The points represent mean values of data obtained in 1982, 1983 and 1984.

Disease-free period (DFP, Table 2). The disease-free period is defined as the period in weeks from planting until the observation of the first mildew symptoms.

1982. In six crops the earliest observation of mildew was immediately after planting. In the districts of Pijnacker and Den Hoorn the mean values of DFP were 12 and 10, respectively, which was shorter than in the other districts. In the districts of Huissen and Northern Netherlands the mean DFP values were higher than in the other districts, viz. 23 and 19, respectively.

1983. In one crop mildew was observed immediately after planting. For most districts the mean DFP values were between 14 and 19. In the districts of Drenthe and Northern Netherlands the mean DFP values were 24 and 22, respectively, which was higher than in the other districts.

1984. In two crops, mildew was observed immediately after planting. The lowest mean DFP value was recorded for the district of Pijnacker, viz. 16. The mean DFP values of the other districts, ranging from 18 to 24, did not differ significantly.

Comparison 1982, 1983, 1984. In 1983 the mean DFP values of Pijnacker, Den Hoorn and Drenthe were higher than in 1982. In 1984 the districts of Barendrecht and East Brabant and Limburg had higher mean DFP values than in 1983. In 1984 the DFP was longer than in 1982 for six districts. Only in the districts of Huissen, Drenthe and Northern Netherlands DFP values were similar to those in 1982.

Apparent rate of increase of infected crops (AIC, Table 3). The AIC is estimated according to Van der Plank (1963).

1982. The AIC value was lowest for the district of Northern Netherlands (0.11), and highest for the district of Pijnacker (0.31). The AIC values for the other districts varied from 0.20 to 0.25.

Table 2. Mean disease-free period (DFP) in weeks in various districts in the Netherlands in 1982, 1983 and 1984.

District	Disease-free period[1]		
	1982	1983	1984
Pijnacker	12 ab[2]	15 a x[3]	16 a x
Den Hoorn	10 a	18 abc x	21 bc x
Barendrecht	14 c	18 abc	22 bc xxx
Huissen	23 e	14 ab	22 bc
Vleuten	14 bc	18 abc	24 c x
East Brabant and Limburg	17 cd	16 ab	19 b xxx
West Brabant	15 bc	19 bc	22 bc x
Drenthe	16 cd	24 d x	18 ab
Northern Netherlands	19 de	22 cd	24 bc
Total	16	18 x	21 xxx

[1] Disease-free period: period in weeks from planting until observation first cucumber powdery mildew.
[2] Values in columns followed by the same letter are not significantly different (p = 0.05).
[3] x: distribution of values is significantly different from that in 1982, xx: distribution of values is significantly different from that in 1983, xxx: distribution of values is significantly different from those in both 1982 and 1983 (Kolgomorov-Smirnov test, p = 0.05).

Table 3. Apparent rate of increase (AIC) of crops infected with cucumber powdery mildew in various districts in the Netherlands in 1982, 1983 and 1984.

District	Apparent rate of increase[1]		
	1982	1983	1984
Pijnacker	0.31	0.28	0.31
Den Hoorn	0.25	0.21	0.16
Barendrecht	0.20	0.24	0.24
Vleuten	0.22	—[2]	—
East Brabant and Limburg	0.24	0.40	0.23
West Brabant	0.23	0.23	—
Drenthe	0.20	0.36	0.21
Northern Netherlands	0.11	0.12	0.07
Total	0.22	0.29	0.26

[1] Linear regression coefficient after logit transformation.
[2] Insufficient data available.

1983. The AIC value for the district of Northern Netherlands was low again, viz. 0.12. The highest AIC value was recorded for the district of East Brabant and Limburg (0.40).

1984. The district Northern Netherlands showed the lowest AIC value (0.07), the district of Pijnacker the highest (0.31).

Comparison 1982, 1983, 1984. AIC values of the district of Pijnacker were constantly high, those of the district of Northern Netherlands were constantly low. Values of the other districts varied between those extremes.

Discussion

Although data obtained by telephone will certainly contain inaccuracies, the good coverage of the inquiry and the intensity with which the growers attend to their crop, guarantee a representative picture of the pattern of appearance of cucumber powdery mildew in the Netherlands.

Survival of conidia is an important factor in the epidemiology of cucumber powdery mildew, particularly because any glasshouse is empty for almost two months. In general, powdery mildew conidia can survive for a period of up to 40 days without a host plant (Blumer, 1967), a period too short to span the gap between two cucumber growing seasons. Perithecia can survive for a longer time. Since perithecia have not yet been found in the Netherlands, their role in the epidemiology of cucumber powdery mildew seems negligible. If conidia surviving the crop-free period in a glasshouse served as the inoculum in that same glasshouse, the symptoms should appear within 14 days after planting. Lesions were only occasionally observed so soon after planting. Even in glasshouses with a heavily diseased crop in the previous year, the new crop can remain mildew-free for months. It is concluded that conidia surviving in a crop-free glasshouse are probably not the primary inoculum.

Since *S. fuliginea* is recorded on many plant species, conidia could possibly survive on plants other than Cucurbitaceae. However, within *S. fuliginea* different pathotypes exist that differ in their ability to attack particular host species (Abiko, 1978, 1982a, 1982b; Boesewinkel, 1979). Thus, infection of cucumber plants with powdery mildew conidia from other plant species seems improbable, although it was suggested to occur in England (Stone, 1962) and Israel (Dinoor, 1974). Moreover, in the Netherlands *S. fuliginea* appears on cucumbers in December/January, when low temperatures outside the glasshouse almost exclude infection from sporulating lesions on non-Cucurbitaceae. The statement that, from a crop-oriented viewpoint, the role of wild hosts in plant disease epidemiology is a minor one, seems appropriate for cucumber powdery mildew (Wheeler, 1981).

If we assume that conidia cannot survive crop-free periods and that cucumber plants can only be infected by a specific form of *S. fuliginea,* then cucumber powdery mildew inoculum can only originate from infected Cucurbitaceae. In the Netherlands, Cucurbitaceae do not occur outside the glasshouse in the winter months. Although the growing season for cucumbers is discontinuous in individual glasshouses, in the Netherlands as a whole cucumber plants are grown year-round (Fig. 1). Especially in the districts of Pijnacker and Den Hoorn, with a high density of production crops and nurseries, cucumber plants are always available for the mildew to grow on. Few conidia will make the step from the old crops to young plants in November/December.

However, the fact that young cucumber plants cannot be adequately treated with fungicides because of phytotoxicity, enables these few conidia to establish infections. Since only few conidia will be able to infect plants in nurseries, much between-glasshouse dispersal will have to take place in order to reach the high level of incidence observed in July and August. The first mechanism of dispersal is by means of planting stock, carrying latent infections, that is transported from nurseries to production glasshouses. Especially when cucumber plants show mildew symptoms immediately or a few weeks after planting, growers impute this phenomenon to infected planting stock. The intensity of this mechanism varies from year to year, possibly influenced by weather, hygienic measures, disease pressure and planting dates.

In districts with a high crop density, such as Pijnacker and Den Hoorn, the probability of infection of young plants will be higher than in other districts. The relatively high occurrence of early infected crops in 1982 in those districts may be attributed to that high crop density.

A second possible mechanism of dispersal is the transportation of conidia from one crop to another by persons visiting glasshouses. The observation of growers, who detected the first mildew symptoms on places where visitors entered the crop, appears to affirm this possibility.

Other parts of the glasshouse where first mildew symptoms were frequently observed, were places with much air circulation. Since glasshouses are not so air-tight as has often been suggested, conidia can probably leave and enter the glasshouses fairly easily, as has been reported for other pathogens (Frinking and Scholte, 1983; Zadoks, 1967; Zandvoort, 1968). The occurrence of first mildew lesions on places with much air circulation was not restricted to districts with a high crop density, but was also observed in districts where crops were situated several kilometers apart. These observations suggest a third mechanism of dispersal, namely the dispersal of conidia by wind.

Significant differences in FOM and DFP values were observed between years. Probably these differences were not caused by one factor only, but by an inextricable complex of factors such as weather, hygienic measures, planting dates, disease pressure and chemical control. In spite of this complexity a pattern can be indicated.

1. In all districts a gradual increase in numbers of infected crops was observed from planting until May in 1982, 1983 and 1984.
2. Mildew was generally observed earlier in districts with a high crop density than in districts with a low crop density.
3. The mean disease-free period was shorter in districts with a high crop density, and longer in districts with a low crop density.
4. The apparent rate of increase of infected crops in the district of Northern Netherlands was lower than in the other districts, whereas the AIC in the district of Pijnacker was always high.

A few general conclusions are ventured. Overwintering of inoculum is possible because cucumber plants are grown all year round. Dispersal of cucumber powdery mildew early in the growing season takes place by transportation of infected planting stock and by visitors. When the disease pressure increases in the course of the growing season, inoculum is dispersed by wind.

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Samenvatting

Een patroon in het verschijnen van komkommermeeldauw in Nederlandse kassen

In 1982, 1983 en 1984 werd een enquête gehouden om informatie te verzamelen over de overleving en verspreiding van komkommermeeldauw. Tuinders die hun gewas geplant hadden in december, januari of februari werd gevraagd wanneer ze de eerste meeldauw hadden waargenomen.

Een geleidelijke toename van het aantal geïnfecteerde gewassen werd waargenomen vanaf het planten tot aan mei. Geïnfecteerde gewassen werden voor het eerst waargenomen direct na het planten. De weeknummers van de eerste meeldauwwaarnemingen, de ziekte-vrije perioden en de snelheden waarmee het aantal geïnfecteerde gewassen toenam, werden berekend voor verschillende districten.

In het district met de grootste gewasdichtheid (Pijnacker) werd de meeldauw over het algemeen vroeg in het groeiseizoen waargenomen, terwijl de snelheid waarmee het aantal geïnfecteerde gewassen toenam hoger was dan in andere districten. In het district met de laagste gewasdichtheid (Noord Nederland) werd de meeldauw laat in het seizoen waargenomen en was de snelheid waarmee het aantal geïnfecteerde gewassen toenam laag.

Mogelijke manieren van overleving en verspreiding van inoculum worden besproken. Er wordt gesuggereerd dat het overwinteren van inoculum mogelijk is, doordat komkommerplanten het gehele jaar aanwezig zijn. De verspreiding van komkommermeeldauw zou plaats kunnen vinden door het vervoer van geïnfecteerd plantmateriaal, bezoekers en wind.

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Persistence of resistance to fungicides in Sphaerotheca fuliginea

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Abstract

Isolates of *Sphaerotheca fuliginea* collected in 1981-1983 in cucumber glasshouses in the Netherlands were tested for their sensitivity to benzimidazole fungicides, dimethirimol, dinocap and pyrazophos.

Resistance to dinocap was not observed, although this fungicide has been used for over 30 years. Resistance to benzimidazole fungicides and dimethirimol has been persistent since these fungicides were withdrawn for control of cucumber powdery mildew more than 10 years ago. Although pyrazophos has only been used incidentally after 1977, the level of resistance has not decreased.

Factors possibly involved in the persistence of resistance and implications for disease control in practice are discussed.

Additional keywords: cucumber powdery mildew, benomyl, carbendazim, dimethirimol, dinocap, pyrazophos.

Introduction

Various fungicides have been used to control cucumber powdery mildew *(Sphaerotheca fuliginea)* in the Netherlands. Among them are sulphur, dinitrophenol derivatives (dinobuton, dinocap), dimethirimol, benzimidazoles (benomyl, carbendazim), pyrazophos and ergosterol biosynthesis inhibitors (EBIs: bitertanol, fenarimol, imazalil, triforine). The years during which they have been used are given in Fig. 1.

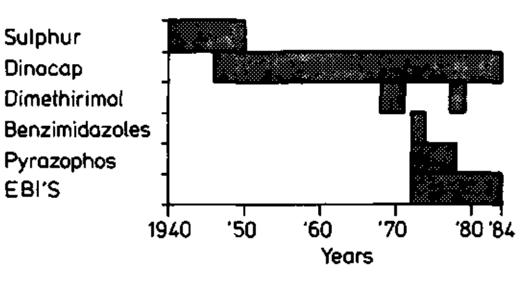

Fig. 1. Periods during which various fungicides have been used intensively in the Netherlands for control of *Sphaerotheca fuliginea*.

Application of dimethirimol and benzimidazole fungicides had to be discontinued after a few years because of development of resistance (Bent et al., 1971; Kooistra et al., 1972). The use of pyrazophos was drastically reduced when *S. fuliginea* developed partial resistance to this fungicide (Dekker and Gielink, 1979). Failure of disease control by EBIs has only been observed for triforine (Schepers, 1983).

Upon cessation of fungicide application after development of resistance, the resistant population can persist or gradually disappear. It was the aim of this study to investigate the persistence of resistance to dimethirimol, two benzimidazoles and pyrazophos in *S. fuliginea* in Dutch cucumber glasshouses. The study was carried out in 1981-1983.

Materials and methods

Plants. Cucumber plants *(Cucumis sativus* L.) cv. Lange Gele Tros were used in the experiments. The plants were grown in plastic pots (ϕ 15 cm) filled with steamed soil under mildew-free conditions in a growth chamber (18 °C, 80% rh, Philips TLMF 40W/35 RS, 16 h a day, 7000 lux) for 3 to 5 weeks.

Sampling. Isolates of *S. fuliginea* were sampled by collecting several diseased cucumber leaves from small areas (1 to 2 m^2) in glasshouses. The isolates were transferred to mildew-free cucumber plants, and subcultured every two weeks. After one or two transfers sufficient conidia were available for testing the sensitivity to the fungicides.

Chemicals. Dinocap (technically pure) and Karathane (22.5% WP dinocap) were generously supplied by Aagrunol B.V., Groningen, the Netherlands; Milcurb (12.5% EC dimethirimol) by ICI Holland B.V., Rotterdam, the Netherlands; carbendazim (technically pure) and Benlate (50% WP benomyl) by E.I. du Pont de Nemours and Co., Wilmington, Delaware, USA and du Pont de Nemours, 's-Hertogenbosch, the Netherlands; pyrazophos (technically pure) and Curamil (30% EC pyrazophos) by Farbwerke Hoechst A.G., Frankfurt, Germany and Hoechst Holland N.V., Amsterdam, the Netherlands.

Stock solutions of dinocap, dimethirimol and pyrazophos were made in methanol and of benomyl and carbendazim in dimethylsulfoxide (DMSO). They were stored at −20 °C and freshly prepared every 4 weeks.

Leaf disc test. Inoculation was carried out by pressing mildew-free leaves onto leaves with the profusely sporulating mildew isolate to be tested. Discs (ϕ 12 mm) were cut with a corkborer from heavily inoculated leaves. The discs were placed on fungicide solutions in Petri dishes (ϕ 5 cm), 5 discs per dish. The final solvent concentration of methanol or DMSO was always lower than 1% and 0.1%, respectively.

In the case of the non-systemic fungicide dinocap, the discs were sprayed with solutions by means of a Potter precision spray tower. Petri dishes were incubated in closed plastic boxes under fluorescent light (Philips TLMF 40W/35 RS, 16 h a day, 7000 lux) at 20 °C for 6-7 days.

Mildew development was recorded, using the following scale: 0, no visible mildew development; 1, 0-5%; 2, 5-25%; 3, 25-50%; 4, 50-75% and 5, more than 75% of disc surface covered with cucumber powdery mildew. EC_{50} values of fungicides for inhibition of mildew development were obtained by intrapolation from the dosage-response curves.

Foliar spray and drench test. Mildew-free cucumber plants, 3 to 4 weeks old, were sprayed to run-off with the fungicides. Fungicide suspensions were made from formulated products. Leaves were allowed to dry and inoculated by bringing them in contact with heavily infected leaves. In drench tests 20 ml of formulated fungicide suspensions were administered to the soil, and thereafter plants were inoculated. The percentage of infected leaf area was assessed after 7 to 10 days of incubation under greenhouse conditions (60-80% rh, 17-23 °C). EC_{50} and EC_{90} values of fungicides for inhibition of fungal growth were determined as described for the leaf disc test.

Results

In October 1981 cucumber powdery mildew isolates were collected in 60 glasshouses distributed throughout the Netherlands. In October 1982 and October 1983 isolates were obtained from 13 glasshouses. All isolates were identified as *S. fuliginea*.

Dinocap. The variation in sensitivities of the test isolates to dinocap was similar to that of the reference isolates (Table 1). This is in agreement with the results of the foliar spray tests in which control of a typical glasshouse isolate of 1982 (D17) was similar to that of the wild-type isolate (Table 2).

Dimethirimol. EC_{50} values of dimethirimol for control of the reference isolates in leaf disc tests were lower than 1 μM, whereas the EC_{50} values of most test isolates were higher. Furthermore, sensitivity of the test isolates was comparable to the sensitivity of isolates tested in 1970, immediately after the first observations on development of resistance to the fungicide (Table 1). The differential sensitivity of reference and test isolates as observed in leaf disc tests was also noticed in drench tests (Table 2).

Pyrazophos. In leaf disc tests the test isolates showed a decreased sensitivity to pyrazophos. Although the number of isolates tested in 1977 is too small to make a valid comparison, there seems to be no indication of a regression of resistance (Table 1). In foliar spray tests glasshouse isolate D17 was still controlled by the recommended rate (Table 2). This is in agreement with the experience of growers who still successfully use pyrazophos in alternation with EBIs. However, its use has drastically been reduced because of incompatibility with biological control of spider mites.

Benzimidazoles. EC_{50} values of carbendazim for control of all test isolates in leaf disc tests were higher than 6 μM. In contrast, reference isolates were eradicated at 0.2-0.5 μM. Kooistra et al. (1972) reported that EC_{50} values of benomyl for control of *S. fuliginea* isolates in leaf disc tests were all considerably higher than 6 μM (Table 1). These findings were confirmed by A.J. Gielink (personal communication). Resistance to benzimidazoles was also obvious in foliar spray tests, since glasshouse isolate D17 could not be controlled at three times the recommended rate of benomyl (Table 2).

Table 1. Sensitivity of *Sphaerotheca fuliginea* isolates from Dutch cucumber glasshouses to carbendazim, dimethirimol, dinocap and pyrazophos.

Fungicide	Year[1]	Number of test isolates per EC_{50} category[2]						
		0-2	2-5	5-10	10-20	20-30	30-60	>60
Dinocap	1981	0	13	76	12	3	0	0
	1982	0	2	19	4	1	0	0
	ref.	0	4	13	2	0	0	0
Dimethirimol	1970[3]	0	7	0	3	0	10	17
	1981	0	2	4	10	24	21	39
	1982	0	0	0	2	1	13	10
	1983	0	0	0	0	11	33	3
	ref	1	10	14	0	0	0	0
Pyrazophos	1977[4]	0	0	0	7	0	0	1
	1981	0	0	10	28	43	11	4
	1982	0	0	0	4	6	15	4
	1983	0	0	0	4	9	27	3
	ref	0	4	21	0	0	0	0
Carbendazim	1972[5]	0	0	0	0	0	0	10
	1981	0	0	0	0	0	0	98
	1982	0	0	0	0	0	0	26
	1983	0	0	0	0	0	0	47
	ref[6]	20	0	0	0	0	0	0

[1] Test isolates were collected in 1981, 1982, 1983.
[2] EC_{50} categories of carbendazim, dimethirimol and pyrazophos in 10^{-7}M and of dinocap in 10^{-5}M.
[3] Data from Bent et al. (1971).
[4] Data from Dekker and Gielink (1979).
[5] Data from tests with benomyl (Kooistra et al., 1972).
[6] Reference isolates have always been maintained in the absence of any fungicide.

Table 2. Sensitivity of a wild-type and a glasshouse isolate (D17) of *Sphaerotheca fuliginea* to fungicides on cucumbers in foliar spray and drench tests.

Fungicide	Isolate			
	wild-type[1]		D17[2]	
	EC_{50}	EC_{90}	EC_{50}	EC_{90}
Dinocap (135)[3]	13.5[4]	80.0	13.5	80.0
Dimethirimol (300)[5]	15.0	90.0	350.0	600.0
Pyrazophos (150)	1.5	15.0	18.0	90.0
Benomyl (250)	7.5	10.0	>750.0	–

[1] Maintained at the Laboratory of Phytopathology, Wageningen, in the absence of fungicides.
[2] Isolate representative for the population of *S. fuliginea* in Dutch glasshouses in 1982.
[3] Between brackets: dosage (mg a.i. l^{-1}) recommended in practice.
[4] Fungicide concentration in mg a.i. l^{-1}.
[5] Between brackets: recommended dosage (mg a.i. l^{-1}) of drench treatment for 5 to 6 weeks old cucumber plants.

Multiple resistance. No isolates were detected which possessed resistance to one fungicide only. Without exception, resistance to carbendazim, benomyl, dimethirimol and pyrazophos were simultaneously present in all isolates. An example is shown in Table 2.

Discussion

Resistance or decreased sensitivity of *S. fuliginea* to benzimidazoles, dimethirimol and pyrazophos was first described in 1972, 1971 and 1979, respectively. The EC_{50} values of benomyl, dimethirimol and pyrazophos for inhibition of mildew growth in leaf disc tests of glasshouse isolates collected in those years were similar to those of the isolates collected in 1981-1983 (Table 1). This indicates that resistance to benzimidazoles and dimethirimol has been persistent for at least 10 years. Resistance to pyrazophos also appears to be stable, but it should be kept in mind that this fungicide, in contrast with the former fungicides, is still used incidentally.

Resistance to dinocap was not observed although this fungicide has been used for more than 30 years. The mutational response needed to circumvent the action of this fungicide, the uncoupling of the oxidative phosphorylation, might be too complex (Lyr, 1977).

Studies on persistence of resistance to dimethirimol and pyrazophos have not been reported in the literature, but strains of several pathogens resistant to benzimidazoles also persisted in the field after cessation of selection pressure (Dovas et al., 1976; Wicks, 1976; Ruppel et al., 1980; Eckert, 1982).

In plant breeding many cases were reported in which pathogen races carrying unnecessary genes for virulence persisted for a variable period of time (Van der Plank, 1968; Parlevliet, 1981). The persistence of resistant insect populations after relaxation of selection pressure was reviewed by Keiding (1967). In some cases the disappearance of pathogen races and insects carrying unnecessary genes was explained by their reduced fitness (Abedi and Brown, 1960; Leonard, 1977).

Such a reduction in fitness has also been observed in fungal strains resistant to fungicides, e.g. to EBIs (cf. Fuchs and De Waard, 1982), dicarboximides (Pommer and Lorenz, 1982), pyrazophos (Dekker and Gielink, 1979), benomyl (cf. Zadoks, 1982) and ethirimol, a fungicide closely related to dimethirimol (Hollomon, 1975, 1978).

If in spite of an observed reduced fitness of fungicide-resistant strains, as was the case with cucumber powdery mildew resistant to pyrazophos (Dekker and Gielink, 1979) and benomyl (cf. Zadoks, 1982), resistance appears to be persistent under natural conditions, several factors might be involved.

1. In the laboratory the fungicide-resistant strains are only selected from a genetically limited sample of the pathogen population, with all consequences thereof (Wolfe, 1982).
2. Under a continuous selection pressure a recovery in fitness might take place. The experience of entomologists is that persistence of resistance is related to age of resistance (Keiding, 1967).
3. Fitness as measured under experimental conditions is rarely identical or even similar to fitness under natural conditions (Parlevliet, 1981; Wolfe, 1982).
4. Intensive chemical control may have fully eliminated the sensitive population, so that resistant isolates do not have to compete with the sensitive wild-type strains. This

hypothesis is supported by the observation that in this survey only few isolates with a wild-type sensitivity were detected (Table 1).

5. Despite a competitive advantage of fungicide-sensitive strains over fungicide-resistant strains, Wild (1980) observed that strains of *Penicillium digitatum* resistant to benzimidazoles did not disappear from the pathogen population in packing houses when benzimidazoles had been replaced by other fungicides. However, many strains resistant to benzimidazoles were multiple resistant to sec-butylamine or sodium o-phenyl-phenate (SOPP). In competition experiments Wild inoculated oranges with a mixture of a wild-type strain and a strain that was resistant to benzimidazoles and sec-butylamine or SOPP. Treatment of the fruit with either benomyl or sec-butylamine or SOPP resulted in an immediate increase in the frequency of the benomyl-resistant strain in the pathogen population. Thus, persistence of resistance to benzimidazoles might be explained by the use of SOPP and sec-butylamine. The multiple resistance of glasshouse isolates of *S. fuliginea* to all systemic fungicides tested, including the EBIs (Schepers, 1983), may also have been responsible for the persistence of resistance to benzimidazoles, pyrazophos and dimethirimol.

The results described in this report reject the idea to reintroduce benzimidazoles and dimethirimol for control of *S. fuliginea* in the Netherlands. This is in analogy with the observation that regression of resistance to insecticides has never been sufficient for an 'old' insecticide to be reintroduced (Conway and Comins, 1979).

The present study confirms Gilpatrick's (1983) statement that once resistance to fungicides appears in practice, fungicide-resistant strains will probably be stable and are likely to persist, at least in low numbers, for an indefinite period of time in the absence of the fungicide. Thus it seems that the period a fungicide can be used effectively is longest if development of resistance to that fungicide is prevented.

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Samenvatting

Persistentie van resistentie tegen fungiciden in Sphaerotheca fuliginea

De gevoeligheid voor twee benzimidazool-fungiciden en dimethirimol, dinocap en pyrazofos werd getoetst van *Sphaerotheca fuliginea* isolaten die in 1981-1983 verzameld waren in komkommerkassen in Nederland. Resistentie tegen dinocap werd niet waargenomen, ofschoon dit fungicide al meer dan 30 jaar wordt gebruikt.

De resistentie tegen benzimidazool-fungiciden en dimethirimol, die meer dan 10 jaar geleden werden teruggetrokken voor de bestrijding van *S. fuliginea,* was persistent. Hoewel pyrazofos slechts incidenteel gebruikt is sinds 1977, is het resistentieniveau niet teruggelopen. Factoren die mogelijk betrokken zijn bij de persistentie van resistentie en de gevolgen voor de ziektebestrijding worden besproken.

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

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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 sensitivity 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 (⌀ 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 germination 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}(μM) 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.

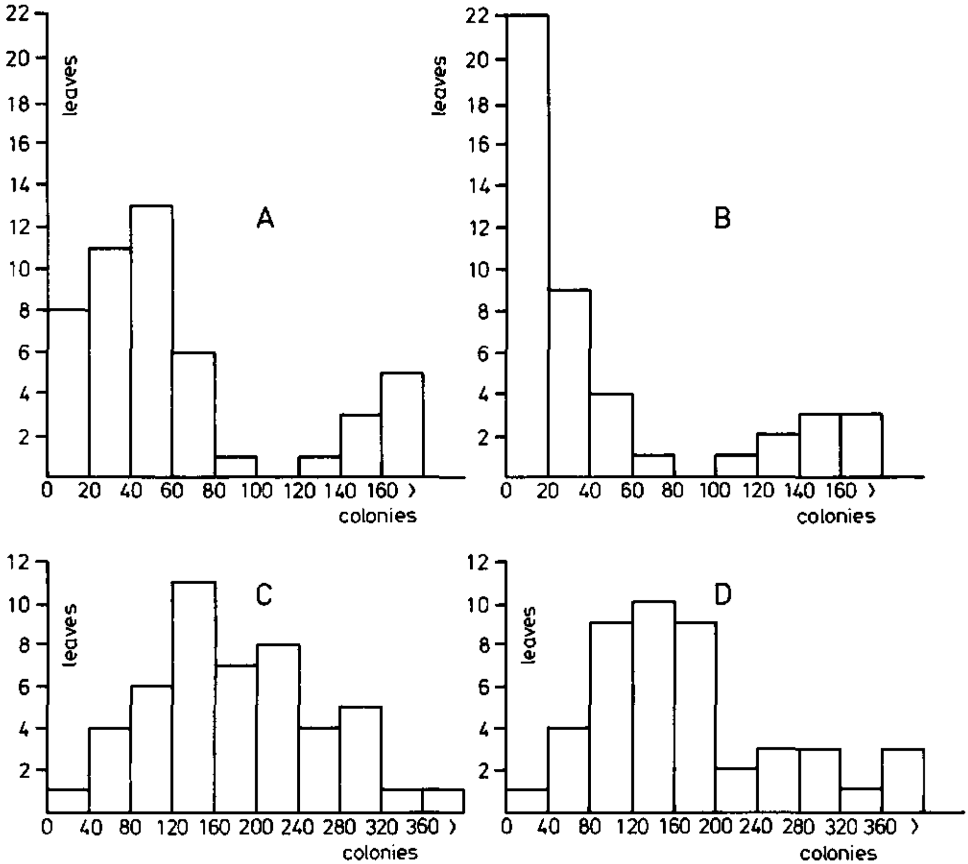

Fig. 1. Frequency distributions of the number of sporulating colonies resulting from inoculation with dry conidia and with conidial suspensions of *Sphaerotheca fuliginea* isolates. Isolates were resistant (R3, R10) or sensitive (S1, S2) to fungicides which inhibit ergosterol biosynthesis.
1) A: conidial 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.

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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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SHORT COMMUNICATION

Decreased sensitivity of Sphaerotheca fuliginea to fungicides which inhibit ergosterol biosynthesis

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

Fungicides which inhibit ergosterol biosynthesis (EBI's) are widely used for disease control in a variety of crops. Most of them are regarded as fungicides with a specific mechanism of action (cf. Ragsdale, 1977). This implies the risk of development of resistance. Indeed, in vitro EBI-resistant strains can be readily isolated (cf. Fuchs and De Waard, 1982). In practice no disease control failure due to EBI resistance has been reported. However monitoring of EBI-treated cereals revealed a decreased sensitivity of powdery mildew to these fungicides (Fletcher and Wolfe, 1981; Hollomon, 1982; Laws et al., 1982; Buchenauer, 1983).

In the Netherlands, EBI's (fenarimol, imazalil, triforine and recently bitertanol) are used for control of cucumber powdery mildew *(Sphaerotheca fuliginea).* Many growers treat their crop almost exclusively with these fungicides; application of ten sprays during one growing season is not exceptional. This spray program gives rise to a high selection pressure which favours build-up of an EBI-resistant pathogen population. Development of resistance of *S. fuliginea* under similar conditions has been described earlier for dimethirimol, benomyl and pyrazophos (Bent et al., 1971; Kooistra et al., 1972; Dekker and Gielink, 1979). For this reason the sensitivities to EBI's of two *S. fuliginea* isolates from EBI-treated crops and a reference isolate were compared.

The reference isolate used has been maintained at the Laboratory of Phytopathology for more than 10 years. It has never been in contact with any fungicide, and is regarded to have a wild-type sensitivity. One test isolate was collected in 1982 in a glasshouse in the Netherlands, where imazalil was the only fungicide used for the previous 2 years. The other isolate was sampled in 1982 from a fenarimol-treated cucumber field in Israel.

Fungitoxicity of the EBI's was determined in a foliar spray test. In this test 3 to 4 week-old cucumber plants were sprayed in duplicate to run-off with formulated fungicide solutions at various concentrations. The leaves were allowed to dry and conidia of the isolate to be tested were rubbed on the leaves. The percentage of leaf area infected with cucumber powdery mildew was assessed after 7 days of incubation under greenhouse conditions (60-80% r.h., 17-23 °C).

The EC_{50} and EC_{90} values of the EBI's tested for the isolates from the Netherlands

Table 1. Sensitivity of *Sphaerotheca fuliginea* isolates form the Netherlands and Israel to ergosterol biosynthesis inhibitors in foliar spray tests with cucumber plants.

Fungicide	Isolate					
	wild-type[1]		D-17[2]		Isr-1[3]	
	EC_{50}	EC_{90}	EC_{50}	EC_{90}	EC_{50}	EC_{90}
Bitertanol (300.0)[4]	1.5[5]	30.0	7.0	70.0	9.0	60.0
Fenarimol (24.0)	0.2	0.5	3.0	5.0	2.5	6.0
Imazalil (50.0)	0.5	4.0	3.5	30.0	15.0	45.0
Triforine (200.0)	6.0	60.0	>600.0	–	>600.0	–

[1] Maintained at the Laboratory of Phytopathology, Wageningen.
[2] Collected in a glasshouse in the Netherlands.
[3] Sampled from a cucumber field in Israel.
[4] Between brackets: dosage (mg a.i. l^{-1}) recommended in practice.
[5] Fungicide concentration in mg a.i. l^{-1}.

Tabel 1. Gevoeligheid van isolaten van Sphaerotheca fuliginea uit Nederland en Israel voor ergosterolbiosynthese remmers in spuitproeven met komkommerplanten.

and Israel were higher than those of the wild-type isolate (Table 1). The recommended dosages of bitertanol and fenarimol were still high enough to achieve a proper control of the EBI-resistant isolates. Control of the resistant isolates by imazalil was marginal only and could just barely be obtained at the recommended dosage. The resistance level to triforine was high and above the recommended dosage. This is in accordance with the experience of growers who apply triforine only at the beginning of the growing season, and later switch to fenarimol and imazalil, because of poor mildew control by triforine.

From 1972 to 1977, when triforine was the only EBI available, it was successfully used during the whole growing season. This may be due to the fact that triforine exerts a low selection pressure: a result of its short half-life in aqueous suspensions (Fuchs and Ost, 1976). The continuous selection pressure of more persistent EBI's (fenarimol, imazalil) may have increased the level of EBI resistance to such an extent that the weakest EBI, triforine, lost much of its efficacy. In discussing resistance to triforine, Fuchs et al. (1977) already mentioned the introduction of more powerful EBI's as a potential hazard to triforine.

The decreased sensitivities to EBI's of the Dutch and the Israeli isolates were almost similar. In the Netherlands a change to shorter spray intervals was sufficient to again achieve proper control by fenarimol and imazalil, contrary, to what has been found in Israel where control provided by EBI's including fenarimol has been impaired in some cases (M. Faulkner, personal communication). This discrepancy may be related to different rates of breakdown of the fungicides in the two countries, due to different climatic conditions (temperature, UV-light intensity). In addition, infection pressure in Israel is relatively high because of rapid spread of conidia by winds in outdoor cucumber crops.

It is concluded that use of EBI's results in selection of *S. fuliginea* isolates with decreased sensitivity to EBI's which may lead to partial or complete loss of the EBI's' ability to control this pathogen. Additional problems of resistance to EBI's of *S. fuliginea* have to be expected when the selection pressure is not drastically reduced. Strategies to reduce selection pressure of EBI's should thus be strongly recommended.

Samenvatting

Verminderde gevoeligheid van Sphaerotheca fuliginea voor fungiciden die de ergosterolbiosynthese remmen

Isolaten van *Sphaerotheca fuliginea* uit Nederland en Israel vertoonden een verminderde gevoeligheid voor de ergosterolbiosynthese remmers bitertanol, fenarimol, imazalil en triforine. In enkele gevallen heeft dit reeds geleid tot het falen van de bestrijding.

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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,

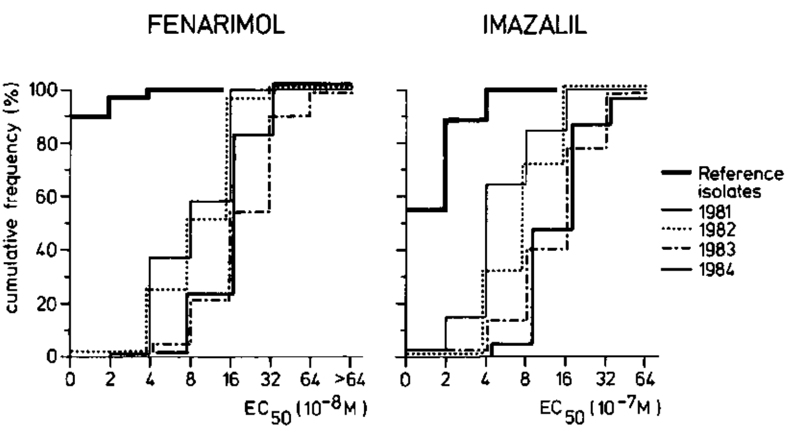

Fig. 1. Cumulative frequency curves of the sensitivity to fenarimol and imazalil of *Sphaerotheca fuliginea* isolates collected from 1981 to 1984 in the Netherlands. The number of isolates tested was 97 in 1981, 75 in 1982, 125 in 1983, and 96 in 1984. During the survey, eight different reference isolates 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

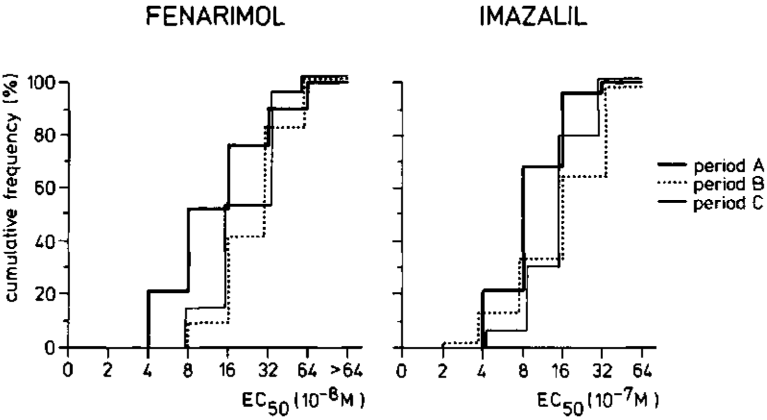

Fig. 2. Cumulative frequency curves of the sensitivity to fenarimol and imazalil of *Sphaerotheca fuliginea* isolates collected in three periods of the growing season of 1983. The number of isolates collected in period A was 28, in period B 45, and in period C 50. The curves for period A differed significantly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

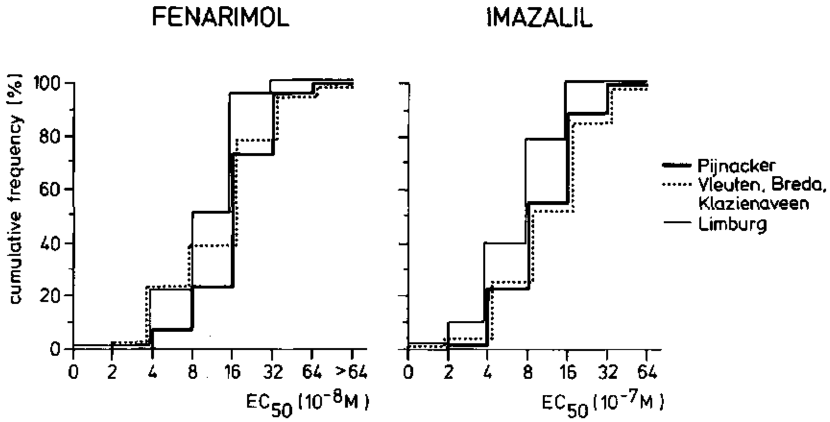

Fig. 3. Cumulative frequency curves of the sensitivity to fenarimol and imazalil of *Sphaerotheca fuliginea* isolates collected in various districts of the Netherlands. The number of isolates collected from 1981 to 1984 in Pijnacker was 154, in Limburg 104, and in Breda, Vleuten and Klazienaveen 134. 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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SUMMARY AND GENERAL CONCLUSIONS

Ergosterol biosynthesis inhibitors (EBIs) have a remarkably broad spectrum of antifungal activity. They belong to the commercial fungicides which exhibit the highest activity known to date. Resistance to EBIs was found in vitro, but the level of resistance and the decreased fitness of resistant strains led to the hypothesis that resistance to EBIs was rather unlikely to develop in practice. Despite the fact that EBIs were introduced as early as 1972, up to 1980 only data on EBI-resistant laboratory strains were available. However, gradually there appeared reports on decreased sensitivity to EBIs in practice. This made it of interest to investigate the potential development of resistance to EBIs in vivo.

In this study the pathogen used to gather the desired information was *Sphaerotheca fuliginea*, as this fungus is widespread in cucumber glasshouses in the Netherlands, intensively controlled with fungicides and resistance-prone. This thesis contains five papers describing several aspects of resistance to fungicides in *S. fuliginea*.

In the first paper it has been shown that *S. fuliginea* is present on cucumber plants all year round. A gradual increase in the number of infected crops, in the form of S-shaped curves, was observed from planting until May. In the districts with the highest crop density (Pijnacker), mildew was generally observed early in the growing season, while the apparent rate of increase of infected crops was higher than in other districts. In the district with the lowest crop density (Northern Netherlands), mildew was observed late in the growing season and the apparent rate of increase of infected crops was low. Early in the growing season the pathogen is probably dispersed by transportation of infected planting stock and by man. When the disease pressure increases, inoculum is probably dispersed by wind. This implies that inoculum is highly mobile and that, in the case of development of resistance to fungicides, the resistant strains will disperse to crops where the spray regime did not cause resistance to develop.

As *S. fuliginea* developed resistance to dimethirimol, benzimidazoles and pyrazophos in 1971, 1972 and 1979, respectively, it was thought that presence of strains resistant to these fungicides might provide information on the long-term fitness of these strains. The results of an investigation are presented in the second paper. Strains resistant to these fungicides still persisted in the pathogen population. Although this finding may indicate that the long-term fitness of these strains is equal to that of wild-type strains, several other factors might be involved in the persistence of resistance.

From 1981 to 1984, *S. fuliginea* in the Netherlands was primarily controlled

with EBIs. The short-term fitness of isolates of *S. fuliginea* resistant to EBIs and their behaviour under practical conditions are described in the third, fourth and fifth paper.

Several fitness parameters of EBI-resistant isolates collected from commercial glasshouses were compared to those 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. The results are described in the third paper. One or more values of fitness parameters for EBI-resistant isolates were significantly lower than those for the wild-type isolates. However, in general it was concluded that the fitness of EBI-resistant *S. fuliginea* isolates, collected in commercial glasshouses, was hardly reduced. Within the group of EBI-resistant isolates tested no significant relation was observed between the degree of resistance to EBIs and the degree of fitness.

In the fourth and fifth paper the resistance to EBIs of *S. fuliginea* over a three-year period has been described. In 1981 the sensitivity of glasshouse isolates to EBIs was lower than that of the wild-type isolates. In 1982 and 1983 the sensitivity decreased further. In 1984, no significant differences in sensitivity with the 1983 level were apparent. Per district, the degree of resistance appeared to be positively correlated to the frequency of application of EBIs. 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.

Triforine showed a very low efficacy in controlling glasshouse isolates. This is in accordance with the experience of growers who, therefore, only use triforine when the disease pressure is low. It is concluded that the decreased sensitivity to triforine can be regarded as resistance. In the case of bitertanol, fenarimol and imazalil, a change to shorter spray intervals has up till now been sufficient to achieve proper control.

Contrary to earlier presumptions that development of resistance to EBIs seemed rather unlikely, it gradually developed in normal commercial practice. The slowly decreasing sensitivity to EBIs of cereal powdery mildew is in line with the results described in this thesis. Strategies to delay resistance to EBIs are necessary. Alternation with other site-specific and multi-site fungicides must be advised and can be applied immediately. Non-EBI fungicides are registered for control of cucumber powdery mildew in the Netherlands. Appropriate information and guidance by extension officers and fungicide manufacturers may lower the reluctance of growers to use these strategies.

In the long run, control of S. *fuliginea* might only be maintained by a form of integrated control, which accepts less than perfect disease control. Biological and chemical control, induced resistance of cucumber plants, epidermal coating and mildew-tolerant cucumber cultivars are elements that may fit into a strategy for integrated control of cucumber powdery mildew.

SAMENVATTING

Ergosterolbiosyntheseremmers (EBR's) hebben een opvallend breed werkingsspectrum tegen schimmels. Zij behoren tot de commerciële fungiciden met de tot op heden hoogste activiteit. Resistentie tegen EBR's werd gevonden in vitro. De resistentiegraad en de verminderde fitness van de resistente stammen leidden tot de veronderstelling dat resistentie tegen EBR's zich in de praktijk waarschijnlijk niet zou kunnen ontwikkelen. In weerwil van het feit dat al in 1972 een vertegenwoordiger van de EBR's geïntroduceerd was, waren er tot 1980 alleen gegevens bekend over EBR-resistente laboratoriumstammen. Geleidelijk verschenen er echter publikaties waarin melding werd gemaakt van een verminderde gevoeligheid voor EBR's onder praktijkomstandigheden. Hierdoor werd het van belang de ontwikkeling van resistentie tegen EBR's in vivo te onderzoeken.

Sphaerotheca fuliginea is gebruikt om die gewenste informatie te verzamelen. Deze schimmel is wijd verbreid in komkommerkassen in Nederland, wordt intensief bestreden met fungiciden en ontwikkelt gemakkelijk resistentie. Dit proefschrift bevat vijf artikelen die verschillende aspecten van resistentie tegen fungiciden in *S. fuliginea* beschrijven.

In het eerste artikel is aangetoond dat *S. fuliginea* het gehele jaar door aanwezig is op komkommerplanten. Een geleidelijke toename van het aantal geïnfecteerde gewassen, in de vorm van S-vormige curven, werd waargenomen vanaf het planten tot aan mei. In het district met de grootste gewasdichtheid (Pijnacker), werd de meeldauw over het algemeen vroeg in het groeiseizoen waargenomen, terwijl de snelheid waarmee het aantal geïnfecteerde gewassen toenam hoger was dan in andere districten. In het district met de laagste gewasdichtheid (Noord Nederland), werd de meeldauw laat in het seizoen waargenomen en was de snelheid waarmee het aantal geïnfecteerde gewassen toenam laag. In het begin van het groeiseizoen wordt het pathogeen waarschijnlijk verspreid door transport van geïnfecteerd plantmateriaal en door mensen. Als de infectiedruk toeneemt, wordt het inoculum waarschijnlijk ook verspreid door wind. Het inoculum is erg mobiel en in het geval van ontwikkeling van resistentie tegen fungiciden zullen de resistente stammen ook verspreid worden naar komkommergewassen waar het spuitregime geen resistentie-ontwikkeling tot gevolg had.

Aangezien *S. fuliginea* resistent werd tegen dimethirimol, benzimidazolen en pyrazophos in respectievelijk 1971, 1972 en 1979, zou het nog immer aanwezig zijn van stammen die resistent zijn tegen deze fungiciden informatie kunnen verschaffen over hun fitness op lange-termijn. De resultaten van dit onderzoek zijn vermeld in het tweede artikel. Stammen met resistentie tegen deze fungiciden kwamen nog steeds in de pathogeenpopulatie voor. Ofschoon dit resultaat erop kan wijzen dat de fitness op lange-termijn van deze stammen gelijk is aan die van wild-type stammen, kunnen nog andere factoren een rol spelen bij de persistentie van resistentie.

Van 1981 tot 1984 werd *S. fuliginea* in Nederland voornamelijk met EBR's bestreden. De fitness op korte-termijn van EBR-resistente isolaten van *S. fuliginea* en hun gedrag onder praktijkomstandigheden worden beschreven in het derde, vierde en vijfde artikel.

Verschillende fitness-parameters van EBR-resistente isolaten die verzameld waren in commerciële komkommerkassen werden 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. De resultaten zijn vermeld in het derde artikel. Een of meer waarden van fitness-parameters van de EBR-resistente isolaten waren significant lager dan die van de wild-type isolaten. Er wordt echter geconcludeerd dat de fitness van EBR-resistente *S. fuliginea* isolaten, verzameld in komkommerkassen in Nederland, nauwelijks verminderd was. Binnen de onderzochte groep van de EBR-resistente isolaten werd geen significante correlatie waargenomen tussen de mate van resistentie en de mate van fitness.

In het vierde en vijfde artikel wordt de resistentie van *S. fuliginea* tegen EBR's gedurende een drie-jarige periode beschreven. In 1981 was de gevoeligheid van de kas-isolaten voor EBR's lager dan die van de wild-type isolaten. In 1982 en 1983 werd een verdere vermindering van de gevoeligheid voor EBR's waargenomen. In 1984 was geen significant verschil met de gevoeligheid in 1983 aantoonbaar. Per district bleek de mate van resistentie positief gecorreleerd te zijn met de frequentie van toepassing van EBR's. 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. Triforine bestreed kas-isolaten erg slecht. Dit is in overeenstemming met de ervaring van tuinders die daarom alleen triforine gebruiken bij een lage infectiedruk. Geconcludeerd wordt dat de verminderde gevoeligheid voor triforine beschouwd kan worden als resistentie. Daarentegen is in het geval van bitertanol, fenarimol en imazalil verkorting van de spuitintervallen tot nog toe voldoende geweest om een goede bestrijding te bewerkstelligen.

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CURRICULUM VITAE

Hubertus Thomas Antonius Maria Schepers werd op 21 juni 1956 geboren in Zevenbergschen Hoek. In 1974 behaalde hij het diploma Atheneum-B aan het Sint Jans College te Den Haag, waarna hij zijn studie aan de Landbouwhogeschool te Wageningen begon. In juli 1980 studeerde hij met lof af in de studierichting planteziektenkunde met als hoofdvak fytopathologie en als bijvakken entomologie en erfelijkheidsleer. Van september 1981 tot september 1984 was hij als promotie-assistent werkzaam bij de vakgroep Fytopathologie van de Landbouwhogeschool. Sinds 1 juni 1985 is hij werkzaam als takcoördinator voor de glasteelten bij het Consulentschap in Algemene Dienst voor de gewasbescherming te Wageningen.