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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 Resistance to benzimidazole fungicides and dimethirimol has been persistent since these fungicides were withdrawn for control of cucumber powdery mildew more than 10 years ago.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.

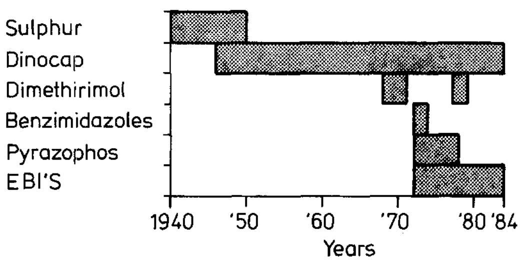

Fig. 1. Periods during which various fungicides have been used intensively in the Netherlands for control of *Sphaerotheca fuliginea*.

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.

References

Abedi, Z.H. & Brown, A.W.A., 1960. Development and reversion of DDT resistance in *Aedes*

aegypti. Can. J. Genet. Cytol. 2: 252-261.
Bent, K.J., Cole, A.M., Turner, J.A.W. & Woolner, M., 1971. Resistance of cucumber powdery mildew to dimethirimol. Proc. 6th Brit. Insectic. Fung. Conf. (Brighton, 1971), Vol. 1, 274-282.
Conway, G.R. & Comins, H.N., 1979. Resistance to pesticides. Lessons in strategy from mathematical models. Span 22: 53-55.
Dekker, J. & Gielink, A.J., 1979. Decreased sensitivity to pyrazophos of cucumber and gherkin powdery mildew. Neth. J. Pl. Path. 85: 137-142.
Dovas, C., Skylakakis, G. & Georgopoulos, S.G., 1976. The adaptability of the benomyl-resistant population of *Cercospora beticola* in Nothern Greece. Phytopathology 66: 1452-1456.
Eckert, J.W., 1982. *Penicillium* decay of citrus fruits. In: Dekker, J. & Georgopoulos, S.G. (Eds), Fungicide resistance in crop protection. Pudoc, Wageningen, p. 231-250.
Fuchs, A. & Waard, M.A. de, 1982. Resistance to ergosterol biosynthesis inhibitors. I. Chemistry and phenomenological aspects. In: Dekker, J. & Georgopoulos, S.G. (Eds), Fungicide resistance in crop protection. Pudoc, Wageningen, p. 71-86.
Gilpatrick, J.D., 1983. Management of resistance in plant pathogens. In: Georghiou, G.P. & Saito, T. (Eds), Pest resistance to pesticides. Plenum Press, New York, p. 735-767.
Hollomon, D.W., 1975. Behaviour of a barley powdery mildew strain tolerant to ethirimol. Proc. 8th Brit. Insectic. Fung. Conf. (Brighton, 1975), Vol. 1, p. 51-58.
Hollomon, D.W., 1978. Competitive ability and ethirimol sensitivity in strains of barley powdery mildew. Ann. appl. Biol. 90: 195-204.
Keiding, J., 1967. Persistence of resistant populations after the relaxation of the selection pressure. Wld. Rev. Pest Control 6: 115-130.
Kooistra, T., Sloot, W.H.A. & Jansen, W.H., 1972. Resistentie van komkommermeeldauw *(Sphaerotheca fuliginea)* tegen Benlate, Topsin M en Bavistin. Gewasbescherming 3: 121-125.
Leonard, K.J., 1977. Selection pressures and plant pathogens. Ann. N.Y. Acad. Sci. 287: 207-222.
Lyr, H., 1977. Effects of fungicides on energy production and intermediary metabolism. In: Siegel, M.R. & Sisler, H.D. (Eds), Antifungal compounds. Marcel Dekker, New York, Vol. II, p. 301-332.
Parlevliet, J.E., 1981. Stabilizing selection in crop pathosystems: an empty concept or a reality? Euphytica 30: 259-269.
Pommer, E.H. & Lorenz, G., 1982. Resistance of *Botrytis cinerea* Pers. to dicarboximide fungicides – a literature review. Crop Protection 1: 221-230.
Ruppel, E.G., Jenkins, A.D. & Burtch, L.M., 1980. Persistence of benomyl-tolerant strains of *Cercospora beticola* in the absence of benomyl. Phytopathology 70: 25-26.
Schepers, H.T.A.M., 1983. Decreased sensitivity of *Sphaerotheca fuliginea* to fungicides which inhibit ergosterol biosynthesis. Neth. J. Pl. Path. 89: 185-187.
Van der Plank, J.E., 1968. Disease resistance in plants. Academic Press, New York and London, 206 pp.
Wicks, T., 1976. Persistence of benomyl tolerance in *Venturia inaequalis*. Pl. Dis. Reptr 60: 818-819.
Wild, B.L., 1980. Resistance of citrus green mold *Penicillium digitatum* Sacc. to benzimidazole fungicides. Doctoral Thesis, Univ. of California, Riverside, 89 pp.
Wolfe, M.S., 1982. Dynamics of the pathogen population in relation to fungicide resistance. In: Dekker, J. & Georgopoulos, S.G. (Eds), Fungicide resistance in crop protection. Pudoc, Wageningen, p. 139-148.
Zadoks, J.C., 1982. Can we use models describing the population dynamics of fungicide-resistant strains? In: Dekker, J. & Georgopoulos, S.G. (Eds), Fungicide resistance in crop protection. Pudoc, Wageningen, p. 149-160.