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Decreased sensitivity to pyrazophos of cucumber and gherkin powdery mildew

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Abstract

In some cucumber and gherkin greenhouses in the Netherlands, where Curamil (a.i. pyrazophos) had been used for control of powdery mildew, unsatisfactory disease control was obtained in recent years. Laboratory tests revealed a decreased sensitivity of the pathogen to the fungicide. The fitness and the competitive ability of pyrazophos resistant strains in absence of the fungicide appeared to be somewhat lower than that of the normal, sensitive strains. The implications with respect to disease control in practice are discussed.

Additional keywords: Curamil, resistance to systemic fungicide, *Sphaerotheca fuliginea.*

Introduction

During the last decennium several systemic fungicides have become available for control of powdery mildew of cucumber and gherkin. Soon after their introduction reports appeared about unsatisfactory disease control with some of these fungicides, e.g. benomyl (Schroeder and Provvidenti, 1969) and dimethirimol (Bent et al., 1971). This appeared due to development of resistance to these fungicides in the causal pathogen, *Sphaerotheca fuliginea* (Schlecht ex Fr.) Poll.

In 1971 Curamil (a.i. pyrazophos) was introduced and in the following years successfully applied for control of powdery mildew in cucumber and gherkin greenhouses. In 1975 and 1976, however, two cases of unsatisfactory disease control with pyrazophos were reported to the Plant Protection Service in the Netherlands; subsequent tests with samples of the pathogen from these greenhouses on young cucumber plants showed that higher doses of the fungicide were required than normally needed for efficient disease control (Kooistra, 1976). The purpose of this research was to investigate whether the decreased effect of pyrazophos was due to development of resistance to this compound, and if so, to study the nature of the resistant strains.

Materials and methods

The cucumber varieties grown in the commercial greenhouses were 'Farbio', 'Suzanne' and 'Uniflora', the gherkin variety was 'Levo'. For control of powdery mildew the growers applied the officially recommended doses of Curamil, an emulsifiable concentrate, containing as active ingredient 300 g/kg of pyrazophos (0,0 - diethyl - 0 - (6-ethoxycarbonyl - 5 - methylpyrazolo (1,5-a) pyrimid - 2 - yl)phosphorothioate).

Samples, containing at least ten leaves with sporulating powdery mildew, were collected between 13 May and 21 October from greenhouses with the following fungicide treatments:

I. No Curamil applied in 1977 or in the preceding years;
II. Curamil applied as the prime control agent in 1977;
III. No Curamil applied in 1977 in view of unsatisfactory results with this compound in the preceding year.

Immediately after the samples were received in the laboratory, the pathogen was transferred to young cucumber plants, cv. Lange Gele Tros. After the onset of sporulation, conidia were harvested for inoculation of leaf discs, 18 mm diam., floating on aqueous solutions of pyrazophos. Development of powdery mildew was assessed after 7-10 days, using a scale from 0 (healthy) to 5 (> 80% of leaf surface covered with mycelium).

Results

The sensitivity of the powdery mildew isolates from the commercial greenhouses was assessed in the leaf disc test. The results are presented in Table 1. From this table it appears that powdery mildew isolates from greenhouses, in which never Curamil had been used, were almost all inhibited at 0.3 ppm of pyrazophos in the leaf disc bioassay, but that isolates from greenhouses with Curamil treatment in 1977 often still grow at 1.0 or even 3.2 ppm of the fungicide. In those greenhouses, where Curamil had been applied in 1976, but not in the following year, the sensitivity of the pathogen had almost reverted to normal.

In order to test the behaviour of resistant strains (R) in competition with sensitive strains (S) in absence of the fungicide, plants were inoculated with various mixtures of conidia; the S : R ratios used were 10 : 90, 50 :50 and 90 : 10. When the leaves were covered with sporulating mycelium, the conidia were harvested and used for inoculation of a new batch of plants. This procedure was repeated five times. After each transfer the ratio between sensitive and resistant colonies was assessed in the following way. Plants were inoculated with a diluted suspension of conidia, harvested from the test plants (250 000 conidia per ml), which resulted in the appearance of widely separated colonies. From these leaves hundred discs, 18 mm diam. and each containing only one mildew spot, were punched out and transferred to a fungicide solution with 1 ppm pyrazophos for discrimination between sensitive and resistant colonies. The results, presented in Table 2 show that, in absence of the fungicide, the ratio S : R changes in favour of the sensitive pathogen population.

In order to test the fitness of resistant and sensitive isolates in absence of the fungicide, the following experiment was carried out in March 1978. Eight young cucumber plants were inoculated with a De Vilbiss sprayer, containing 5 ml of a spore suspension, 200 000 conidia/ml, of a pyrazophos-resistant isolate. Disease development was compared with that on control plants inoculated in the same manner with conidia of a normal, sensitive strain. After 18 days the powdery mildew colonies were counted on 12.6 dm^2 of leaf surface in each treatment. The number of colonies of the resistant isolate was 156/dm^2, and that of the sensitive pathogen strain 275/dm^2; moreover, development of the pyrazophos-resistant colonies appeared slightly slower than that of the normal colonies.

Table 1. Sensitivity to pyrazophos of powdery mildew from greenhouses with different fungicide treatments. Leaf disc bioassay.

Category	Location	Grower	Date of sampling	cv.	Fungicide	Disease index[1] (treatment in ppm)				
						0	0.3	1.0	3.2	10
I[1]	Huissen	C	10 Aug.	Uniflora[2]	Morestan 2x	5	0	0	0	0
			6 Sept.	,,	Morestan 2x	5	0	0	0	0
			30 Sept.	,,	Benlate 1x	5	0	0	0	0
	Den Hoorn	K	13 May	Farbio[2]	Karathane 1x	5	1	0	0	0
			23 June	,,	Funginex 4x	5	0	0	0	0
			11 Aug.	,,	Funginex 1x	5	0	0	0	0
II[1]	Huissen	H	19 July	Uniflora[2]	–	5	0	0	0	0
			6 Sept.	,,	Curamil 3x	3	0	0	0	0
			30 Sept.	,,	Curamil 4x	4	3	1	1	0
	Den Hoorn	K	3 June	Farbio[2]	Curamil 1x	5	2	0	0	0
			23 June	,,	Curamil 2x	5	3	1	0	0
			11 July	,,	Curamil 2x	5	3	1	0	0
	Wernhout	L	23 Sept.	Levo[3]	Curamil2x	3	3	1	0	0
			7 Oct.	,,	Curamil 2x	3	3	1	0	0
			20 Oct.	,,	Curamil 2x	4	3	3	3	0
	IJsselmuiden	D	17 June	Uniflora[2]	Curamil 2x Funginex 2x Derosal 2x	5	3	1	0	0
III[1]	Harmelen	J	14 July	Farbio[2]	Karathane 1x	5	1	0	0	0
			7 Sept.	,,	Plondrel 1x	4	1	0	0	0
	Belfeld	C	9 June	Levo[3]	–					
			10 Aug.	,,	Morestan 4x Topsin 3x	5	2	0	0	0
	IJsselmuiden	W	1 July	Uniflora[2]	–	5	0	0	0	0
			25 Aug.	,,	Derosal 1x Funginex 5x Benlate 2x	5	0	0	0	0

[1]See Materials and methods.
[2] cv. of cucumber.
[3]cv. of gherkin.

Tabel 1. Gevoeligheid voor pyrazofos van meeldauw uit kassen met verschillende fungicidebehandelingen.

This experiment was repeated with the same pyrazophos-resistant isolate in December 1978, thus under less favourable light conditions. In addition to the number of colonies which developed, also the size of the colonies and the degree of sporulation was measured (Table 3).

From these experiments it appears that the fitness of the pyrazophos-resistant strains was significantly lower than that of the normal sensitive strain, and that the difference in fitness between resistant and sensitive isolates even increased, when they were kept separately during 1978 in the greenhouse of the laboratory.

The level of pyrazophos resistance in the isolates obtained from the commercial greenhouses was never very high. In order to test the possibility of an increase in the

Table 2. Competition between pyrazophos-resistant (R) and -sensitive (S) isolates of *Sphaerotheca fuliginea* on cucumber plants in absence of the fungicide. Percentage of resistant isolates in the mixtures after each of 5 transfers on a new batch of plants.

Initial ratio S : R	Transfer No.	Resistant isolates (%)				
		1	2	3	4	5
	After	20	40	69	93	103 days
90 : 10		9	7	6	1	2
50 : 50		50	34	24	18	6
10 : 90		85	81	77	28	16

Tabel 2. Competitie tussen pyrazofos resistente (R) en gevoelige (S) isolaten van Sphaerotheca fuliginea op komkommerplanten in afwezigheid van het fungicide. Percentage resistente isolaten in de mengsels na elk van 5 transfers op een nieuwe serie toetsplanten.

Table 3. Fitness of pyrazophos-resistant (R) and sensitive (S) isolates of *Sphaerotheca fuliginea* on cucumber plants, in absence of the fungicide.

	R	S
number[1] of colonies/dm^2	5.3	42.4
diameter[2] of colony in cm	7.4	9.3
conidia[3] produced/cm^2	1.4×10^4	3.4×10^4

[1]Average of 12.6 dm^2.
[2]Average of 30 colonies.
[3]Average of 20 x 0.5 cm^2.

Tabel 3. Vitaliteit van pyrazofos resistente (R) en gevoelige (S) isolaten van Sphearotheca fuliginea op komkommerplanten, bij afwezigheid van het fungicide.

level of resistance, three of the resistant isolates were transferred six times successively to discs floating on solutions with an increasing concentration of pyrazophos, namely 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 ppm. Per treatment four leaf discs were used. In none of these cases growth of the pathogen at concentrations above 3 ppm was observed.

Discussion

Unsatisfactory control of cucumber- and gherkin powdery mildew with Curamil in some greenhouses in 1977 appeared due to development of resistance of *Sphaerotheca fuliginea* to this fungicide.

In experiments in vitro Georgopoulos et al. (1975) failed to obtain mutants of *Ustilago maydis*, resistant to 2-hydroxy-5-methyl-6-ethoxycarbonylpyrazolo (1,5-α) pirimidine, the toxic principle responsible for the action of pyrazophos. In experiments in vitro with *Pyricularia oryzae* De Waard (pers. comm.) obtained strains with a moderate, but significant degree of resistance to pyrazophos.

Emergence of resistance in vitro, however, does not necessarily mean that resistance problems will arise in practice; the latter will depend on the fitness of the fungicide resistant strains, the selection pressure by the fungicide and environmental conditions (Dekker, 1977). It was noticed that the sensitivity of the pathogen population reverted to normal after application of Curamil was stopped. In a laboratory experiment with one of the resistant isolates, it was found that the competitive ability of this isolate, in absence of the fungicide, was somewhat lower than that of the normal sensitive pathogen.

This might be due to instability of the resistance, or to a decreased fitness of the resistant isolate, as was earlier found for pimaricin resistant isolates of *Cladosporium cucumerinum* (Dekker and Gielink, 1979). From the present experiments it appeared that in absence of the fungicide, the pyrazophos-resistant isolate was less fit than the original, sensitive pathogen. Although a reduced fitness will hamper the development of resistant strains, a pathogen population with moderate resistance to pyrazophos apparently could build up in greenhouses where Curamil was used. The following factors may have played a role:

1. A moderate selection pressure during prolonged periods, which eliminated the highly sensitive individuals and thus created an environment with less competition for the resistant strains, so that even those with decreased fitness could survive.
2. A high sporulation capacity of the pathogen, which facilitates the spread of resistant strains by conidia.
3. A more or less isolated environment (greenhouse) with relatively little opportunity for entrance of conidia from outside.

Two possibilities might be considered to counteract the development of pyrazophos resistance in practice:

1. An increase of the selection pressure by the fungicide to such a level, that also the resistant strains are killed; this might be attempted in view of the relatively moderate level of resistance.
2. A combined or alternate use of pyrazophos with other fungicides with a different mechanism of action.

The first method would only be successful if no strains with a much higher level of resistance would develop; moreover, a strong increase of the fungicide dose should be feasible technically and economically, and without exceeding the residue tolerance level. In view of this the second method probably would be preferable, since it would interrupt the selection pressure by pyrazophos, and provide an opportunity for competition by individuals sensitive to this fungicide.

Samenvatting

Afgenomen gevoeligheid voor pyrazofos van komkommer- en augurkemeeldauw

In enkele kassen, waar Curamil (a.i. pyrazofos) in 1977 aangewend werd ter bestrijding van meeldauw op komkommers en augurken, werd onvoldoende effekt met dit middel tegen deze ziekte verkregen. Toetsing in het laboratorium bracht een verminderde gevoeligheid van het pathogeen voor het fungicide aan het licht (Tabel 1). Bij afwezigheid van pyrazofos bleek het competitief vermogen (Tabel 2) en de

vitaliteit (Tabel 3) van de pyrazofos resistente isolaten wat lager te zijn dan die van het normale, gevoelige pathogeen. De betekenis hiervan met betrekking tot de ziektebestrijding wordt besproken.

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