Neth. J. Pl. Path. 84 (1978) 27–34

Chemical control of Didymella bryoniae in cucumbers

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Accepted 24 June 1977

Abstract

The effect of a number of fungicides on mycelial growth of *Didymella bryoniae* on agar was tested and compared with the effect on inoculated plants and in commercial crops. Activities in vitro and vivo are not always correlated. Spraying of inoculated young plants is a reliable and quick method for testing fungicides against *D. bryoniae*.

In commercial crops the treatments in decreasing order of effectiveness were weekly sprays of benomyl 0.025 – 0.05 % active ingredient (a.i.), chlorothalonil 0.15 % and triforine 0.02 % a.i. These chemicals should be sprayed alternately to prevent the development of strains tolerant to benzimidazole derivates.

Introduction

Didymella bryoniae (Auersw.) Rehm causes a variety of symptoms in cucumber (*Cucumis sativus* L.) grown under glass in the Netherlands. Leaves, stems, growing tips and fruits can all be attacked. Especially infection of the fruits causes economic losses. Often fruits decay during storage and handling after harvest (Veenman, 1972). Sometimes the fungus attacks melon (*Cucumis melo* L.) and gherkin (*Cucumis sativus* L.) in the Netherlands. Many other species of the cucurbit family can be infected (Chupp and Sherf, 1960).

The disease is known as black fruit rot or gummy stem blight. Many synonyms of the pathogen are used in the phytopathological literature, most frequently *Mycosphaerella citrullina* (C.O. Sm.) Gross. and *Mycosphaerella melonis* (Pass.) Chiu & Walker. The correct names are *Didymella bryoniae* (Auersw.) Rehm for the perfect state and *Phoma cucurbitacearum* (Fr.) Sacc. for the imperfect state (Boerema and Van Kesteren, 1972).

Dithiocarbamates are usually recommended for control (Anonymus, 1975; Fletcher and Preece, 1966), but results are often unsatisfactory. For that reason Naaldwijk Station has tested several chemicals under different circumstances. So it was possible to check the correlation between results in vitro and in vivo. We also investigated whether fungicides used for the control of powdery mildew, *Sphaerotheca fuliginea* (Schlecht. ex Link) Pollacci, had any side-effects on *D. bryoniae*.

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Materials and methods

Mycelial growth in vitro. Aqueous suspensions of commercial products of the fungicides (Table 1) were added to molten cherry decoction agar after cooling to about 45 °C. Concentrations of 0, 1, 10 and 100 μg active ingredient (a.i.) per millilitre were tested. The agar plates were inoculated with 5 mm discs of agar with *D. bryoniae*. Colony diameters were measured after incubation for five days at 20 °C.

Trials with young plants. Cucumber plants of cultivar Uniflora D were potted in 12 cm plastic pots three days after sowing and were inoculated when the second leaf had a diameter of 5 cm. Per plant, about 4 ml suspension of 10^6 conidia/ml was sprayed with a Sprayon spray-set. The fungicides (Tables 2 and 3) were sprayed at a concentration of 250 μg a.i. per ml and at the concentration normally recommended, either 24 h before or 24 h after inoculation. There were six plants per treatment in each of four replicates. The plants were incubated in the greenhouse under a transparent plastic tent at a temperature fluctuating between 20 and 30 °C and a relative humidity almost always of 100 % for the first seven days. For the next seven days the plastic was removed and the relative humidity fluctuated between 40 and 90 %.

Trials in commercial crops: Weekly sprayings. Cultivar Uniflora D was planted at the beginning of July 1974. Three and a half months later, this autumn crop was cleared. The crop was sprayed weekly with the fungicides mentioned in Table 4, for the first time 10 days after planting. The first two times, 0.2 liter/m^2 was sprayed with a mist-sprayer at a pressure of 4–5 atm. Afterwards 0.4 liter/m^2 was used, with fourteen sprays in all. An exception was dimethirimol, for which the soil was drenched six weeks after planting when the first spots of powdery mildew appeared. The number of plants per treatment was 24 in each of two replicates. The temperature was set thermostatically at 18 °C. During the day, higher temperatures were reached with sunshine.

Weekly and fortnightly sprayings. The most promising fungicides were tested with different frequencies in 1975. Cultivar Uniflora D was planted at the end of July. Two months afterwards, when the first attack of *D. bryoniae* was seen, the crop was first sprayed as described earlier. The dimethirimol treatment was applied once at that time. In the weekly scheme, crops were sprayed six times and in the fortnightly scheme three times. The number of plants per treatment was 16 in each of four replicates, each in a separate but identical greenhouse. The temperature was set initially at 20 °C and the setting was lowered after two months to 17 °C. During the day, the temperature rose higher with sunshine.

Results

Mycelial growth in vitro. Results are summarized in Table 1. The growth of the mycelium is inhibited to a certain extent by all the fungicides except dimethirimol. Best results were with benomyl.

Trials with young plants. One week after inoculation, the infection of the second and third leaf of each plant was rated from 0 = healthy to 2 = severely attacked. The

Table 1. Effect of some fungicides on mycelial growth of *D. bryoniae* on cherry decoction agar (means of the sum of two perpendicular diameters of three isolates in three replicates, in mm).

Active ingredient	Proprietary name	Content a.i. and formulation	Concentration in μg a.i./ml		
			1	10	100
benomyl	Benlate	50% w.p.	58	+[1]	0
triforine	Funginex	20% e.c.	77	23	+
dinocap	Karathane	43% e.c.	53	32	10
chloraniformethan	Imugan	25% e.c.	95	68	25
pyrazophos	Curamil-Hoe 2873	30% e.c.	94	80	25
chlorothalonil	Daconil 2787	73% w.p.	63	56	41
zineb	AAphytora	70% w.p.	87	77	58
dinobuton	Acrex-S	48.5% e.c.	78	62	61
quinomethionate	Morestan	25% w.p.	93	75	60
dimethirimol	Milcurb	12.5% e.c.	86	93	93
control (water)			92	92	92

[1] Growth very restricted, not measurable.

Tabel 1. Invloed van verschillende fungiciden op de groei van het mycelium van D. bryoniae op kersagar (gemiddelden van twee loodrecht op elkaar staande diameters van drie isolaten in drie herhalingen, in mm).

Table 2. Effect of some fungicides in a concentration of 250 μg a.i./ml on attack of young cucumber plants one week after inoculation with a conidium suspension of *D. bryoniae* (six plants per treatment; four replicates).

Active ingredient	Sprayed 24 hours before inoculation		Sprayed 24 hours after inoculation	
	% healthy plants	disease index[1,2]	% healthy plants	disease index[1,2]
benomyl	0	2.1[a]	71	0.3[p]
triforine	0	2.5[ab]	25	1.1[pq]
chlorothalonil	0	2.5[ab]	0	1.5[qr]
dinocap	0	2.6[ab]	0	2.0[qr]
zineb	0	3.0[ab]	4	2.0[qr]
chloraniformethan	0	3.0[ab]	0	2.0[qr]
pyrazophos	0	3.1[ab]	0	2.5[rs]
quinomethionate	0	3.1[ab]	0	2.5[rs]
dinobuton	0	3.0[ab]	0	3.2[s]
control	0	3.3[b]	0	3.3[s]

[1] Mean of the sum of the infection rates of the second and third leaf per plant; each leaf rated from 0 = healthy to 2 = severely attacked.
[2] Means marked with different letters differ significantly at $P < 0.05$.

Tabel 2. Invloed van verschillende fungiciden in een dosering van 250 μg a.i./ml op de aantasting van jonge komkommerplanten een week na inoculatie met een sporensuspensie van D. bryoniae (zes planten per behandeling; vier herhalingen).

Table 3. Effects of some fungicides in the normally recommended dosage on attack of young cucumber plants one week after inoculation with a conidium suspension of *D. bryoniae* (six plants per treatment; four replicates).

Active ingredient	μg a.i. per ml	Sprayed 24 hours before inoculation		Sprayed 24 hours after inoculation	
		% healthy plants	disease index[1,2]	% healthy plants	disease index[1,2]
benomyl	500	17	1.7[a]	75	0.3[p]
chlorothalonil	1460	4	2.4[ab]	29	0.9[p]
triforine	200	0	2.5[ab]	0	2.1[qr]
zineb	1400	0	3.0[b]	17	1.2[pq]
dinocap	135	0	3.2[b]	0	2.2[qr]
chloraniformethan	82.5	0	2.9[b]	0	2.4[rs]
pyrazophos	120	0	3.2[b]	0	2.7[rs]
quinomethionate	75	0	3.2[b]	0	2.8[rs]
dinobuton	485	0	3.4[b]	0	2.9[rs]
control		0	3.3[b]	0	3.3[s]

[1] See Table 2.
[2] Means marked with different letters differ significantly at $P < 0.05$.

Tabel 3. Invloed van verschillende fungiciden in de normaal aanbevolen dosering op de aantasting van jonge komkommerplanten een week na inoculatie met een sporensuspensie van D. bryoniae (zes planten per behandeling; vier herhalingen).

disease index was calculated as the mean of the sum of the leaf infection rates per plant (Tables 2 and 3). Two weeks after inoculation, the infection of the second, third and fourth leaf and of the growing tip was rated. These data are not given, as the effects of the treatments were nearly the same as one week after inoculation, although the disease increased and the number of healthy plants decreased in the second week after inoculation.

When sprayed after inoculation, benomyl, chlorothalonil and zineb in both concentrations and triforine 250 μg/ml were significantly better than before inoculation ($P < 0.05$). Triforine and zineb in the highest concentration gave better results than the lower ones ($P < 0.05$). There was no significant difference with concentration of the other fungicides. Dinocap at 250 μg/ml, nearly twice the recommended concentration, was phytotoxic; it caused growth reduction and necrotic leaves.

Trials in commercial crops: Weekly sprayings. The first symptoms of the disease, lesions of black fruiting bodies on the stubs left after removal of fruits or leaves, were seen six weeks after planting out. The main stem can be infected through these stubs. The lesions with black fruiting bodies (pycnidia and perithecia) on the main stem were counted and measured at the end of the crop. As there was a correlation between the number and surface, only the number of lesions per plant is given in Table 4.

The fruits were harvested twice a week and any suspect fruit was cut in half lengthwise to check for internal rot (Fig. 1), which is sometimes hard to see on the outside of the fruit. Samples showed that less than 1 % of the fruits judged healthy were infected. A week after symptoms were seen on foliage, the first fruit with internal rot was found.

Table 4. Effect of weekly sprays with fungicides on *D. bryoniae* in commercial crops (24 plants per treatment; two replicates). A = mean number of lesions per plant on the main stem; B1 = percentage internally rotted fruits; B2 = percentage externally rotted fruits.

Active ingredient	µg a.i./ml	A[2]	B1	B2
benomyl	500	0.3[a]	0.5	7.5
triforine	200	1.2[a]	3.5	16.5
zineb	1400	3.1[b]	5.6	11.1
dinocap	135	4.0[b]	5.8	20.8
quinomethionate	75	4.3[b]	3.7	25.0
dimethirimol	0.25[1]	3.3[b]	3.4	20.5
control		3.4[b]	4.9	5.1

[1] ml active ingredient per plant.
[2] Means marked with different letters differ significantly at $P < 0.05$.

Tabel 4. De invloed van wekelijkse bespuitingen met fungiciden onder praktijkomstandigheden op de aantasting van D. bryoniae (24 planten per behandeling; twee herhalingen). A = gemiddeld aantal lesies op de hoofdstengel; B1 = percentage vruchten met inwendig rot; B2 = percentage vruchten met uitwendig rot.

This rot occurred mostly in the last three weeks. The percentage of fruits with internal rot over the period starting from the first attacked fruit in this trial is given in Table 4.

The picked fruits from the last harvest were kept in boxes covered with plastic at a temperature of about 25 °C. The number of fruits per treatment varied from 63 to 80. After 7 and 14 days, the fruits were checked for external rot (Fig. 2, Table 4).

Weekly and fortnightly sprayings. The data on infection of the stem and fruit were collected in the same way as described above and the results are given in Table 5.

Fig. 1. Internal rot at the blossom end of a cucumber fruit, caused by *D. bryoniae*.

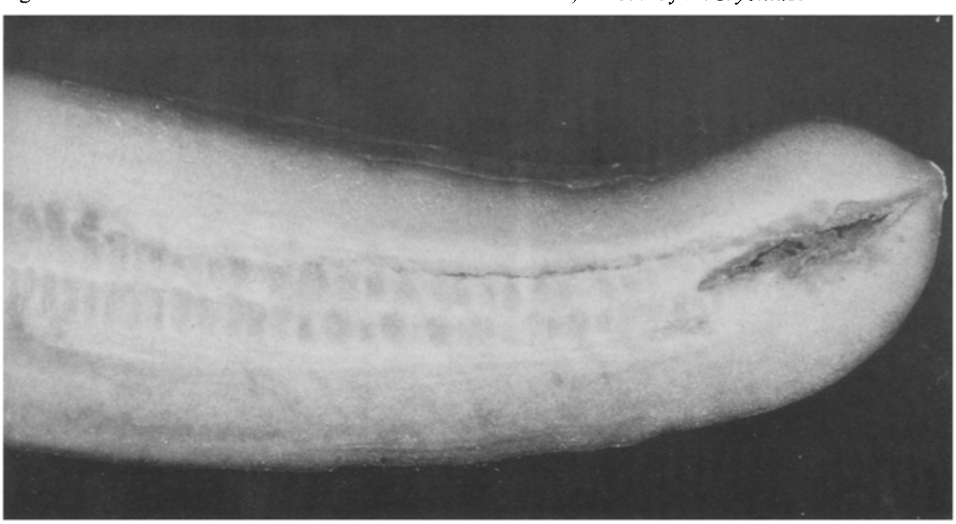

Fig. 1. Inwendig rot bij het bloemeinde van een komkommervrucht veroorzaakt door D. bryoniae.

Three times the picked fruits were kept under humid circumstances to check for external rot. The number of fruits per treatment varied between 64 and 116. Infection of the stem varied in extent. Still the effects of all treatments differ significantly from that with dimethirimol ($P < 0.01$). Weekly sprays of the fungicides differed significantly from fortnightly ones ($P < 0.05$), except for zineb. Internal and external fruit infection is best controlled with benomyl sprayed in a weekly scheme.

Fig. 2. External rot of a cucumber fruit, caused by *D. bryoniae*.

Fig. 2. Uitwendig rot bij een komkommervrucht veroorzaakt door D. bryoniae.

Table 5. Effect of weekly and fortnightly sprays with fungicides on *D. bryoniae* in commercial crops (16 plants per treatment; four replicates). A = mean number of lesions per plant on the main stem; B1 = percentage internally rotted fruits; B2 = percentage externally rotted fruits.

Active ingredient	μg a.i./ml	Frequency	A[2]	B1	B2
benomyl	250	weekly	1.0[a]	2.1	2.2
benomyl	250	fortnightly	1.8[b]	4.0	17.0
chlorothalonil	1460	weekly	0.8[a]	6.1	12.9
chlorothalonil	1460	fortnightly	1.5[b]	5.3	10.9
triforine	200	weekly	1.1[a]	3.5	19.8
triforine	200	fortnightly	1.9[b]	4.2	26.7
zineb	1400	weekly	1.6[b]	8.8	26.5
zineb	1400	fortnightly	1.8[b]	5.4	9.9
dimethirimol	0.25[1]		3.6[c]	4.1	26.9

[1] ml active ingredient per plant.
[2] Means marked with different letters differ significantly at least at $P < 0.05$.

Tabel 5. De invloed van wekelijkse en tweewekelijkse bespuitingen met fungiciden onder praktijkomstandigheden op de aantasting van D. bryoniae (16 planten per behandeling; vier herhalingen). A = gemiddeld aantal lesies op de hoofdstengel; B1 = percentage vruchten met inwendig rot; B2 = percentage vruchten met uitwendig rot.

Discussion

Results with benomyl and triforine are good both in vitro and in vivo. Those with chlorothalonil are better in vivo than in vitro, but with dinocap the opposite was found. Dinocap can be toxic to plants under certain conditions. Results with young plants are closely correlated with those in commercial crops. With inoculation of young plants, fungicides can be tested reliably for their effect on *D. bryoniae* in a simple way, on a small surface and in a short time.

It is difficult to explain why spraying after inoculation gives better results than spraying before inoculation. For control of *Didymella chrysanthemi* (Tassi) Garibaldi & Gullino with chlorothalonil, the opposite was found (Van Steekelenburg, 1976).

An effect on *D. bryoniae* of dinocap, chloraniformethan, pyrazophos, quinomethionate, dinobuton and dimethirimol in the normally recommended dosages for control of powdery mildew, could not be demonstrated (Tables 3 and 4).

There was an interaction between *D. bryoniae* and powdery mildew in the trials in commercial crops. A heavy attack of powdery mildew kills many leaves. The circumstances of a dry microclimate favouring powdery mildew are unfavourable for infection with *D. bryoniae*. A heavy attack of powdery mildew did occur in the control, less in the fortnightly treatments with chlorothalonil and zineb and in the last weeks of the experiments also with dimethirimol. So the strange results of *D. bryoniae* control with these treatments can be explained (Tables 4 and 5). Best results were obtained with benomyl followed by chlorothalonil and triforine. The good results with benomyl may be connected with the mechanism of action and high stability of methyl benzimidazole carbamate (MBC), the fungicidal principle. Though benomyl and triforine are both systemic fungicides, they differ in their mode of action; benomyl has an antimitotic activity (Davidse, 1973) and triforine interferes with sterol synthesis (Sherald and Sisler, 1975). Chlorothalonil is known as a surface protectant and its fungicidal action is attributed to thiol inactivation (Vincent and Sisler, 1968).

With most fungicides, the control of fruit infection is rather disappointing. The proportion of internally rotten fruits was lower than 6% in nearly all treatments. In some cases about 25% of the picked fruits rotted externally. An equal percentage was sometimes found in certain lots on the market (unpublished data). So external rot seems to cause more economic losses than internal rot.

Protection with a good fungicide is difficult to achieve because of the continuous production of wounds and the dense growth of a cucumber crop. The crop must be sprayed nearly every week to have reasonable effect. The life cycle of *D. bryoniae* can be very short. Four days after inoculation, sporulating pycnidia were present under ideal conditions of tests with young plants. Frequent sprays with benomyl or other fungicides producing MBC increase the incidence of strains tolerant to MBC. *Didymella chrysanthemi*, a fungus closely related to *D. bryoniae*, illustrates this phenomenon (Van Steekelenburg, 1973; Grouet, 1974). So benomyl, triforine and chlorothalonil have to be sprayed alternately for control of *D. bryoniae*. In this way, other diseases like powdery mildew, *Botrytis* and *Sclerotinia* will be controlled too.

Samenvatting

Chemische bestrijding van Didymella bryoniae in komkommers

Een aantal chemische middelen zijn in vitro en op verschillende manieren in vivo getoetst op hun werking tegen *Didymella bryoniae*. De resultaten in vitro zijn niet altijd positief gecorreleerd met die in vivo (Tabel 1 en 2). De resultaten na inoculatie van jonge plantjes komen wel overeen met die onder praktijkomstandigheden (Tabel 3, 4 en 5). Op jonge plantjes kunnen dus middelen op een snelle wijze en in een beperkte ruimte betrouwbaar worden getoetst.

Onder praktijkomstandigheden werd het beste resultaat verkregen met benomyl 0,025–0,05 % actieve stof, zowel wat betreft de gewas- als de vruchtaantasting. Hierop volgde chloorthalonil 0,15% a.s. en triforine 0,02% a.s. (Tabel 4 en 5). Indien *D. bryoniae* met chemische middelen moet worden bestreden, is het noodzakelijk dat er bijna elke week wordt gespoten (Tabel 5). Teneinde het ontstaan van resistentie van schimmels tegen benomyl en andere MBC-producerende fungiciden te voorkomen, wordt aanbevolen om benomyl, chloorthalonil en triforine afwisselend toe te passen. Met dit schema worden vele andere schimmelziekten in komkommer eveneens bestreden.

Acknowledgments

Thanks are due to S. J. Paternotte for his technical assistance, to B. J. van der Kaay and P. J. J. Jakobs for carrying out the statistical analyses and to J. C. Rigg for correcting the English text.

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