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Effects of granular nematicides on growth and microbial antagonism to Rhizoctonia solani

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Abstract

Effects of nematicides on growth and microbial antagonism to *Rhizoctonia solani* were investigated as part of a study on the mechanisms involved in the increased incidence of this pathogen in nematicide-treated potato crops.

Ethoprophos inhibited mycelial growth of *R. solani* on potato dextrose agar (PDA), Czapek Dox agar (CDA) and on water agar (WA). Aldicarb stimulated its growth on PDA up to 14% but not on CDA and WA. Oxamyl inhibited mycelial growth on CDA and WA, but not on PDA.

Ethoprophos and aldicarb stimulated development of the mycoparasite *Verticillium biguttatum* on cultures of *R. solani*. The effect was dependent on the medium on which the host fungus was grown. For *Rhizoctonia* cultures on PDA, growth of the mycoparasite was highly promoted by aldicarb and to a lesser extent by ethoprophos. When *R. solani* was grown on CDA, the development of the mycoparasite was not affected by aldicarb, slightly stimulated by ethoprophos and slightly inhibited by oxamyl. On water agar, its development on the host mycelium was not affected.

In field trials on sandy soil, nematicides encouraged *V. biguttatum* probably by increased availability of substrate (i.e. *Rhizoctonia mycelium*) perhaps through reduced activity of the mycophagous fauna.

Soil fungistasis was increased by ethoprophos and to a lesser extent by aldicarb at very high doses. At normal field rates, no effects can be expected on fungistasis. So the increased stem and stolon infection of potatoes in nematicide-treated fields was not caused by a direct effect of the nematicides on growth of *R. solani* or by suppressing the microbial antagonism.

Additional keywords: adsorption, mycoparasitism, side-effects, *Verticillium biguttatum*, aldicarb, ethoprophos, oxamyl.

Introduction

Granular nematicides are applied to minimize yield loss by potato cyst nematodes and other plant-parasitic nematodes. However the use of these nematicides can have a negative side-effect. Several authors reported an increase of infection by *Rhizoctonia solani* Kühn after application of granular nematicides. In field trials, Hide and Corbett (1974) and Leach and Frank (1982) observed an increase in stem infection of potatoes after application of aldicarb. Scholte (1987) reported an increase in stem and stolon infection of potatoes after application of aldicarb, oxamyl or ethoprophos on both marine clay and sandy soil. This was confirmed in trials of Hofman (manuscript in

preparation) on sandy soil.

Ruppel and Hecker (1982) found an increase in *Rhizoctonia* infection in beet after application of aldicarb. The phenomenon has also been noticed for some other pathogens, e.g. increased root rot of snapbeans by *Fusarium roseum* and *F. solani* (Sumner, 1974) and of cucumber by *F. oxysporum* (Sumner, 1978) in fields treated with ethoprophos.

After application of aldicarb to beet, Tisserat et al. (1977) found an increase in damping-off by *R. solani* in steamed soil. However the applied dose was much higher than recommended for field soil.

Several authors reported a fungicidal activity of ethoprophos. Rodriquez-Kabana et al. (1976) found that ethoprophos suppressed infection of peanuts by *Sclerotium rolfsii.* In vitro, mycelium growth of both *R. solani* and *S. rolfsii* was inhibited by ethoprophos. However, the nematicide did not reduce the infection by *R. solani* in the field. The phytotoxicity of ethoprophos reported by Sumner (1974) may increase susceptibility of host plants to infection by *R. solani.*

Bunt (1975) did not find any toxic effects of oxamyl on a range of fungi and bacteria in vitro. Mathur et al. (1980) noticed a stimulation of the microbial population after applying oxamyl at recommended rates to soil.

To understand at which stages of the infection process the nematicides can have an influence, the disease cycle of *R. solani* has to be considered first. The pathogen survives in the form of pseudosclerotia or as mycelium in the soil. In field soil, the sclerotia are kept dormant by soil fungistasis. The growth of *R. solani* may be induced by plant exudates. Hyphae grow over the underground plant parts. At certain places on stems and stolons, infection cushions can develop. In the Netherlands, the type of *R. solani* that causes stem infection belongs in general to the anastomosis group AG-3. Roots are not susceptible to infection by *R. solani* AG-3.

The pathogen causes lesions or, with severe infection, pruning of stems and stolons. Lesion size is dependent on the size of infection cushions, i.e. the amount of mycelium that has developed on the sprout surface (Hofman and Jongebloed, manuscript in preparation).

Organisms that impose dormancy on sclerotia or reduce mycelial growth in soil by secretion of fungistatic compounds may be more sensitive to the toxicity of nematicides than *R. solani* (Bollen, 1979). Therefore effects of nematicides on soil fungistasis had to be studied.

In the Netherlands, by far the most effective mycoparasite reducing vitality of sclerotia of *R. solani* and reducing stem infection is *V. biguttatum* (Van den Boogert and Jager, 1984; Jager and Velvis, 1984). Therefore, this was the only mycoparasite tested for sensitivity to nematicides.

A study was set up to explain the increase in *Rhizoctonia* infection after application of granular nematicides. In this paper, the direct effects on growth of *R. solani* are described, as well as the effects on the microbial antagonism to this pathogen. Studies on the effects on the host-parasite relationship and on interactions of the mycophagous soil fauna with *R. solani* will be reported in subsequent papers.

Materials and methods

Field trials. In 1986, two field trials were set up. One at a light sandy soil (pH 5.2,

content of organic matter 7.1% in Hijken, Province of Drente) and another at a marine clay soil (pH 7.3, content of organic matter 3.1% and 25% clay, 55% silt and 20% sand, in Swifterband, Eastern Flevoland). The previous crops were sugar beet and winter wheat, respectively.

On the sandy soil, the granular nematicides ethoprophos, rate of a.i. 10 kg ha^{-1} (applied as Mocap 20GS), aldicarb, rate of a.i. 3 kg ha^{-1} (as Temik 10G gypsum) and oxamyl, rate of a.i. 5 kg ha^{-1} (as Vydate 10G) were tested. Plot size was 6 m × 10 m. The experimental design was a randomized block with four replicates.

On the clay soil, the nematicides ethoprophos and aldicarb were tested both at recommended rates (50 kg Mocap 20G per ha and 30 kg Temik 10G per ha) and three times as much.

The nematicides were worked into the soil using a spring-tine cultivator on sandy soil and an oscillating harrow on clay soil.

The nematicides were applied on the same day as the potatoes were planted, 22 April on sand and 24 April on clay. The potato cultivars used were 'Prominent' on sand and 'Lady Rosetta' on clay. In order to kill tuber-borne sclerotia of *R. solani*, the seed tubers were treated by dipping in a solution of validamycine (Solacol) with 0.9 g a.i. per l.

Sclerotium initiation. To initiate formation of sclerotia on tubers, haulms were cut (as is done in Dutch seed-potato production to avoid aphid infestation) according to the method described by Dijst (1985). This was done with 30 plants in each plot on sand on 23 July and on clay 21 July. The harvests were three weeks after haulm cutting.

For the final harvest on sand (25 September), no additional haulm destruction was needed for sclerotia initiation, because the crop had died early September from drought. On clay, haulms were destroyed with dinoseb in oil (Chimac, 5 kg a.m. per ha) on 15 September. The final harvest was 22 days later.

Fungi. All tests were set up with *R. solani* AG-3. The isolate is pathogenic for potato and originated from a sclerotium on a tuber from a crop on sandy soil (isolate 05AHa, kindly provided by G. Jager, Institute for Soil Fertility, Haren). *R. solani* was cultured on potato dextrose agar (PDA) unless stated otherwise.

V. biguttatum isolates Gasselte 4 and Haren 17 were kindly provided by G. Jager and P.H.J.F. van den Boogert. Isolate Wildekamp 1 was isolated from one of our own experimental fields. All isolates originated from parasitized sclerotia on potato tubers from sandy soil.

Cultures of *V. biguttatum* were maintained on a medium with 15 g malt extract, 5 g mannitol, 2.5 g yeast extract and 12 g agar in 1 litre distilled water (MMYA).

Plate tests. Direct effects of the nematicides on *R. solani* were determined in a plate test by measuring mycelial growth on agar media supplied with the nematicides in various concentrations. Three media with different nutrient contents were used to study effects of the nematicides on growth of *R. solani*. The media used were potato dextrose agar (PDA, Merck), Czapek Dox agar (Oxoid) and water agar (Oxoid).

Nematicides were tested as their granular formulations: aldicarb as Temik 10G, oxamyl as Vydate 10G and ethoprophos as Mocap 10GS (carrier cepeolite). Concentration ranges were made by first adding 1 g of granules to 0.5 ml ethanol in order to sterilize the granulate. This resulted in 0.05% ethanol in the agar with the highest nematicide

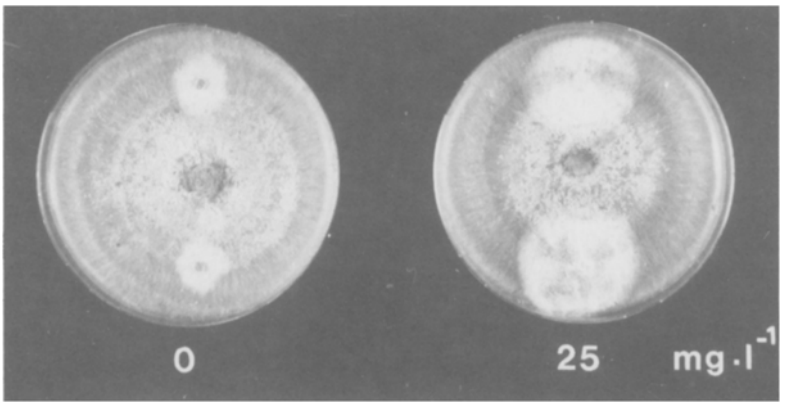

Fig. 1. Effect of aldicarb on the mycoparasitism of *Verticillium biguttatum* on *Rhizoctonia solani*.

concentration. Radial growth was measured on each of five plates of 20 ml agar in 9-cm diameter Petri dishes. The plates were incubated at 20 °C for 72 to 126 h, depending on the test. Growth was expressed as radius of the colonies relative to colonies grown on agar without nematicides.

Effects in vitro on mycoparasitism. The plate test was also used to follow the development of the mycoparasite *V. biguttatum* on *R. solani*. On 7-day-old plates of *R. solani* on PDA, Czapek Dox or water agar, disks of diameter 3mm were placed of a 14-day-old culture of *V. biguttatum* on MMYA (Fig. 1). Growth was measured after incubation for 14 days at 20 °C.

In the tests with ethoprophos and oxamyl, only isolate Gasselte 4 was used, except for the test on PDA in which isolate Wildekamp 1 was used. The test with aldicarb was set up with all three isolates.

Effects in vivo on mycoparasitism. Pieces of stolon, 2 cm in length, were taken from plants from the field trials (50 pieces per plot). These pieces were placed on cultures of *R. solani* on PDA (10 pieces per plate) to allow growth of the mycoparasites on the mycelium (Jager et al., 1979). Plates were incubated for 14 days at room temperature.

The activity of mycoparasites was also studied by examining sclerotia, obtained from tubers at the August and September harvests. Fifty sclerotia from each plot were incubated on moist perlite (without nutrients) in order to induce germination of the sclerotia and subsequent development of mycoparasites on them. After 14 days, germination was examined by rating into four classes: 0, no hyphae formed; 1, one to four hyphae formed; 2, five to twelve hyphae formed and 3, more than twelve hyphae formed. Development of mycoparasites was observed with a dissecting microscope (magnification up to ×100). The most frequently found mycoparasites (*V. biguttatum, Gliocladium roseum* and *G. catenulatum*) were counted.

Fungistasis tests. To investigate whether fungistasis in the soil was affeced by the nematicides, three experiments were set up.

Experiment 1. Shortly after planting of potatoes, sclerotia (c. 2 mm × 3 mm × 1 mm) were buried for 41 days in nylon bags (pore size 1.0 mm) on a depth of 10 cm in the sandy soil field (on 7 May 1985). The sclerotia had been produced on PDA plates. In each plot, 10 bags were buried, each with 10 sclerotia. Germination after burial was rated as in the previously described trial. After 20 days, the ungerminated sclerotia were placed on PDA plates with *R. solani*, in order to examine whether mycoparasites had killed the sclerotia. Growth of mycoparasites was examined 14 days after transfer of the sclerotia.

Experiment 2. Soil samples from the experimental fields were tested in the laboratory for fungistasis by a method described by Davet (1976), who derived the method from Williams and Willis (1962). Soil saturated with water agar was separated from *R. solani* by a cellophane membrane (Cuprophane, 150 P, 12 μm). Before placing a disk with mycelium of *R. solani* on the cellophane, the soil plates covered with cellophane had been incubated for 2 days at 4 °C to give some exchange of substances through the membrane. Inoculation was with disks 3 mm in diameter from five-day-old cultures of *R. solani* on PDA. The plates were subsequently incubated at 16 °C. Soil from each plot was tested for fungistasis in five replicates. When the first colonies reached the edge of the membrane, the colony diameter and hyphal density were measured at a distance of 1 cm from the inoculum disk. This was after about two and half days.

Experiment 3. The sandy soil was treated in the laboratory with different concentrations of the nematicides. Nematicides were applied in aqueous solution to fairly dry soil at 25 ml kg^{-1} soil. Contents of active ingredient of 0, 25, 50, 100 and 250 mg kg^{-1} were obtained in this way. The 100-g samples of soil were divided over five Petri dishes and incubated at room temperature. The test procedure was further the same as for Experiment 2.

Comparing growth on PDA covered with cellophane with growth directly on PDA indicated the diffusion of ethoprophos through the cellophane. The adsorption of ethoprophos in the sandy soil could be calculated by comparing growth rates of *R. solani* on autoclaved soil and on PDA with the different concentrations of nematicide covered with cellophane.

Results

Direct effects on growth of R. solani. The average growth rates of *R. solani* on media without nematicides were 9.27 mm d^{-1} on PDA, 9.03 mm d^{-1} on Czapek Dox and 6.38 mm d^{-1} on water agar.

Aldicarb showed a slight stimulation (14%) of growth of *R. solani* with concentrations of nematicide in PDA agar higher than 5 mg l^{-1} (Fig. 2). When nutrients were limited or not available (Czapek Dox agar or water agar), there was no effect from aldicarb on the growth of *R. solani*.

Oxamyl had no effects on growth in a medium rich of nutrients (PDA). When less nutrients were available (Czapek Dox agar and water agar), it was fungitoxic. With oxamyl at 100 mg per l of Czapek Dox agar, growth of *R. solani* was reduced by 26%. On water agar, the effect was even stronger and the EC_{50} was 55 mg l^{-1}.

Ethoprophos was the most fungitoxic nematicide tested. The EC_{50} of ethoprophos

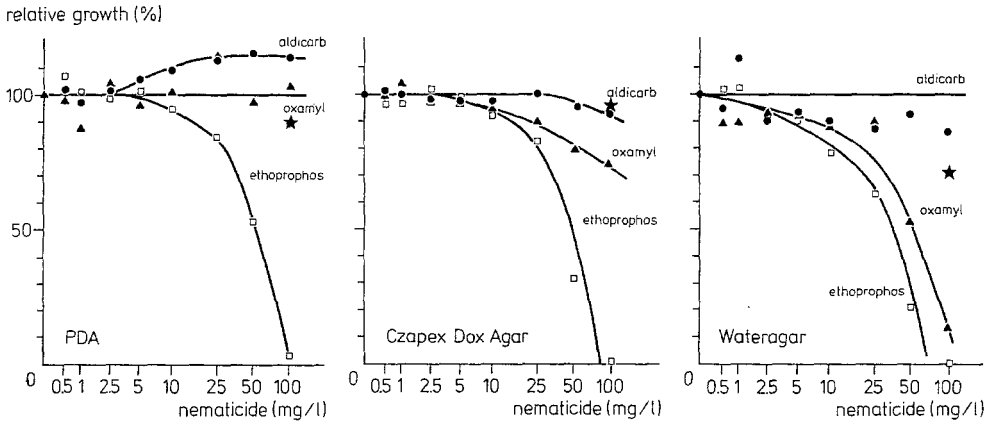

Fig. 2. Effects of nematicides on the relative radial growth of *Rhizoctonia solani* colonies on PDA, Czapek Dox agar or water agar. Growth is expressed as percentage of growth on agar without the nematicides. *, Growth at 0.05% ethanol, which corresponds to the concentration in the plates with the highest nematicide rate.

was 55, 49 and 37 mg l^{-1} on PDA, Czapek Dox agar and water agar, respectively. When technical ethoprophos was used instead of the granulate, the same EC_{50} was found with Czapek Dox agar.

The EC_{50} mentioned for oxamyl and ethoprophos on water agar should actually be corrected, because there was also a fungitoxic effect of the ethanol at these concentrations (Fig. 2). At the maximum concentration of ethanol (0.5 ml l^{-1}) growth was reduced by 33%. However oxamyl and ethoprophos were fungitoxic also without ethanol.

Effects on mycoparasites. The growth rates of *V. biguttatum* on *R. solani* on media without nematicides were 0.28 and 0.40 mm d^{-1} on PDA and Czapek Dox agar, respectively.

The mycoparasitic and saprophytic growth of *V. biguttatum* was influenced by the nematicides tested (Fig. 1, 3 and 4). Aldicarb was the most effective one. A concentration of 25 mg l^{-1} or more in PDA increased growth of *V. biguttatum* on *Rhizoctonia* plates with more than 2.5 times that of the control (Fig. 3). This effect cannot only be attributed to a direct stimulation of growth, which is maximally 33% on PDA (Fig. 4). Ethoprophos stimulated the mycoparasitism on PDA by 60% with 5 and 10 mg l^{-1}, but at higher concentrations (50 mg l^{-1}), growth of *V. biguttatum* on *R. solani* was reduced. However this could also have been caused by limited development of *R. solani* at these concentrations (Fig. 2). Oxamyl did not influence the mycoparasitism.

Surprisingly the effect of aldicarb on mycoparasitism was very limited when the host fungus was grown on Czapek Dox agar (Fig. 3), while also the peak effect of ethoprophos was less than on PDA. There seemed to be a negative linear relation between the growth rate on *Rhizoctonia* plates and natural logarithm of the oxamyl concentration in Czapek Dox agar. On water agar, none of the nematicides induced any effect on the mycoparasitism.

The three isolates of *V. biguttatum* did not differ in their response to aldicarb (Fig.

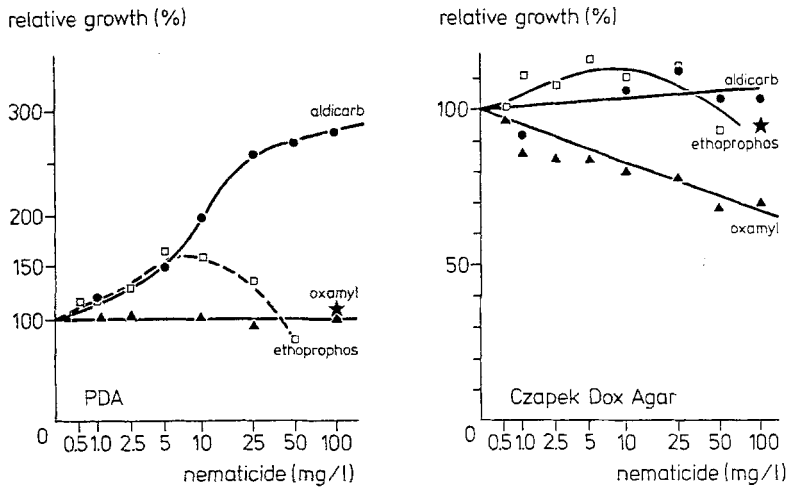

Fig. 3. Effects of nematicides on the relative radial growth of *Verticillium biguttatum* on *Rhizoctonia solani* cultures on PDA or on Czapek Dox agar. *, See Figure 2.

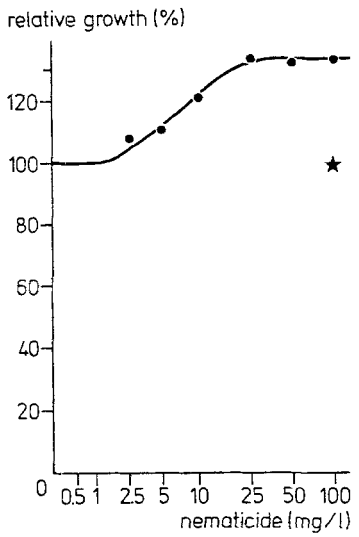

Fig. 4. Effect of aldicarb on the growth of *Verticillium biguttatum* on PDA.

5). Parasitism of all three isolates was highly stimulated on PDA, but growth was only slightly stimulated (14%) on Czapek Dox agar.

Effects on incidence of mycoparasites on stolons and sclerotia and the viability of these sclerotia. On the sandy soil with the June harvest, only 0.5% of the pieces of stolons were occupied by *V. biguttatum*, 6% by *Gliocladium roseum* and 3% by *G. catenulatum*. High incidence of *V. biguttatum* on stolons was only found with the July harvest (Table 1). The incidence of *G. roseum* with the second harvest was 3% and of *G. catenulatum* 1%. The nematicides had not affected the incidence of *G. roseum* and *G. catenulatum*,

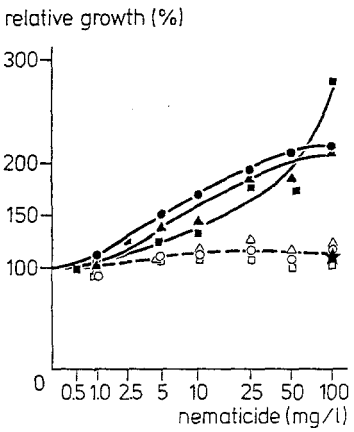

Fig. 5. Effect of aldicarb on the relative radial growth of three isolates of *Vericillium biguttatum* on colonies of *Rhizoctonia solani* on PDA (•–•–•) or Czapek Dox agar (○–○–○). Isolate Haren 17 (■, □) Gasselte 4 (▲, △) and Wildekamp (•,○).

Table 1. Effects of nematicides on the incidence (%) of *Verticillium biguttatum* on stolons and sclerotia in a sandy soil. F_0, soil not fumigated; F_+, soil fumigated with metham-sodium in autumn 1985.

Field	Treatment	Stolons		Sclerotia	
		17 June	30 July	13 August	25 Sept.
F_0	control	0.5	20	16	16
	ethoprophos	—	—	37	45
	aldicarb	1.0	41	57	46
	oxamyl	—	—	58	37
F_+	control	0.0	27	17	12
	ethoprophos	—	—	7	29
	aldicarb	0.5	55	22	8
	oxamyl	—	—	24	27

Differences between treatments were not significant ($P = 0.10$) when using Dunnetts procedure.

Table 2. Incidence (%) of *Gliocladium roseum* and *G. catenulatum* on stolons at the second harvest (21 July) on clay soil. N, recommended rate; 3N, three times recommended rate.

Treatment	*G. roseum*	*G. catenulatum*
control	16	6
aldicarb N	34	7
aldicarb 3N	37	10
ethoprophos N	13	2
ethoprophos 3N	14	4
critical values[1]	13.7	6.7

[1] Dunnetts procedure ($P = 0.05$).

Table 3. Germination index and proportion (%) of sclerotia ungerminated after 41 days of burrying in the field.

Treatment	Germination index[1]	Proportion not germinated (%)
control	50	12
ethoprophos	37	25
aldicarb	48	9
oxamyl	47	19

[1] Germination index $= \frac{\text{not germinated} \times 0 + \text{class } 1 \times 1 + \text{class } 2 \times 2 + \text{class } 3 \times 3}{\text{total number of sclerotia} \times 3} \times 100.$

Differences between treatments were not significant ($P = 0.10$) when using Dunnetts procedure.

but *V. biguttatum* was stimulated by the nematicides. However, the effect was not significant at $P = 0.10$, due to a large difference between replicates.

In clay soil, no mycoparasites were found with the first harvest. With the second harvest, *G. roseum* was found on 23% and *G. catenulatum* on 6% of the stolons (Table 2). On stolons from this trial, *V. biguttatum* was never found. The facultative mycoparasite *Gliocladium roseum* was stimulated by aldicarb, but not by ethoprophos.

The nematicides did not affect the survival of sclerotia in field soil (Table 3). On less than 1% of the sclerotia that had been buried, sporulation of *V. biguttatum* was observed.

Effects on soil fungistasis. Fungistasis in field soils was not affected by nematicide treatments.

The experiment with the concentration range of nematicides in field soil in vitro gave the following results. Oxamyl had no effect on soil fungistasis to *R. solani* (Fig. 6).

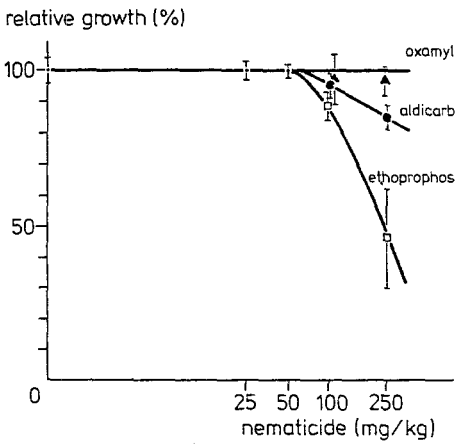

Fig. 6. Effect of nematicides on fungistasis in sandy field soil expressed by relative radial growth on cellophane. Bars represent the standard deviation.

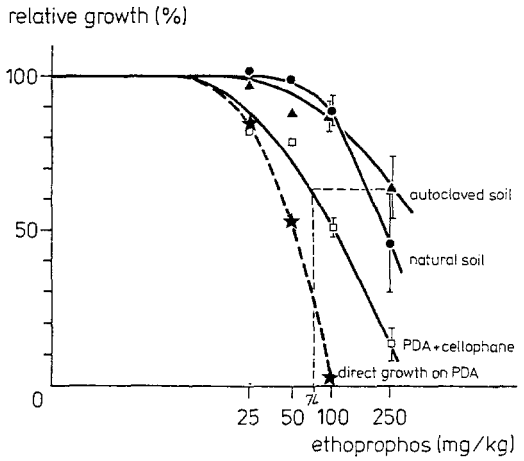

Fig. 7. Effects of ethoprophos on the relative radial growth of *Rhizoctonia solani* in fungistasis tests with different substrates. Bars represent the standard deviation.

At very high rates aldicarb caused an increase in fungistasis. This could not have been due to a direct toxic effect, because on Czapek Dox agar with aldicarb at 250 mg l^{-1} growth was not reduced. Ethoprophos reduced growth at 250 mg kg^{-1} by 54% (EC_{50} of ethoprophos in this sandy soil was 233 mg kg^{-1} soil). This was due to the fungitoxic activity of the product and not to an effect on soil fungistasis, because the fungistasis in unsterilized soil was not significantly higher than in autoclaved soil (Fig. 7).

The cellophane membrane on the medium reduced the inhibition of the test fungus by ethoprophos. The EC_{50} on PDA with cellophane was found with 105 mg l^{-1} in agar and on PDA without cellophane with 53 mg l^{-1} in agar.

A calculation of the adsorption to the soil fraction in the medium can be made with the following equation for the concentration of nematicides in soil:

$$c_{med} = c_l \cdot (\varepsilon_l + \varrho_b \cdot K_{s/l}) \qquad \text{(Leistra, 1977)}$$

where c_{med} is mass concentration of the nematicide in the medium, mg l^{-1}; c_l is mass concentration of nematicide in water phase, mg l^{-1}; ε_l is volume fraction of liquid, l (liquid)/l (medium); ϱ_b is bulk density of soil, kg (soil) l^{-1} (medium); $K_{s/l}$ is adsorption coefficient of nematicide in soil [mg kg^{-1} (soil)]/[mg l^{-1} (liquid)].

When the equation was worked out for ethoprophos at a content of 250 mg kg^{-1} we obtained the following data (1 kg soil and 1 l agar had together a volume of 1.5 l):

c_{med} = 250 mg/1.50 l c_l = 74 mg l^{-1} (Fig. 7)

ε_l = 1.15 l water/1.50 l ϱ_b = 0.85 kg/1.50 l

From these data it can be calcultated that $K_{s/l}$ = 2.62 l kg^{-1}. When the calculation is made at the content of 100 mg of ethoprophos in 1 kg of soil, $K_{s/l}$ = 3.35 l kg^{-1}. The calculated values correspond fairly well with values found by Leistra and Smelt (1981) and their findings that adsorption coefficients decrease slightly with increasing concentrations in the soil.

In a normal field situation, where the granulate is worked into the upper 5 cm of soil, the content of ethoprophos is, however, 15 mg kg^{-1} (about 5 mg l^{-1} in the water phase). Effects on soil fungistasis cannot be expected at this level.

Discussion

Direct effects of the nematicides on the growth of *R. solani* do not imply an increase in incidence of stem infection in the field. Ethoprophos has a fungicidal activity. Contents in field soil can however not be expected to be higher than about 15 mg kg^{-1}. In the sandy soil, this is equivalent to about 5 mg l^{-1} in the water phase. At this concentration, only very small effects on the growth of *R. solani* can be expected. Because aldicarb, oxamyl and ethoprophos all increase stem infection in the field to the same extent (Hofman, manuscript in preparation), direct effects in field soil on mycelial development are unlikely. This implies also that neither the fungitoxic effects of ethoprophos and oxamyl nor the growth-promoting effect of aldicarb play a role at field rates of the nematicides.

The EC_{50} for ethoprophos on PDA (55 mg l^{-1} is somewhat higher than the 30 mg l^{-1} found by Rodriguez-Kabana et al. (1976).

The stimulatory effect of aldicarb on mycelium growth, which was reported by Spurr and Sousa (1974), was confirmed in our trials. Spurr and Sousa only found growth stimulation when a suitable carbon source was available. The slight growth inhibition of aldicarb, which was reported by Ruppel and Hecker (1982) was not in accordance with these results.

Oxamyl was found to be fungitoxic when nutrients were limited for growth of *R. solani*. This explains why Bunt (1975) did not find fungitoxic effects, because he carried out his tests on PDA or malt agar, which are media rich of nutrients.

Nematicides did not adversely affect the mycoparasitism. On PDA, aldicarb and to a lesser extent ethoprophos at lower concentrations stimulated growth of *V. biguttatum* on *Rhizoctonia* colonies. This phenomenon could be due to either a weakening of the resistance of the fungal mycelium to parasitism or a direct growth stimulation of the mycoparasite (Fig. 5). When tested at the same time, different isolates of *V. biguttatum* responded equally to aldicarb. However, the response of an individual isolate was not always the same in different tests.

It is not yet understood why the effects did not occur on Czapek Dox agar. A difference in nutrient supply to *R. solani* seems to cause different effects of aldicarb on the susceptibility to mycoparasitism.

Effects on the incidence of mycoparasites on stolons did not appear to the same extent in the two experimental fields. Incidence of mycoparasites on stolons is strongly dependent on three factors: substrate availability (i.e. *Rhizoctonia mycelium*), presence of propagules of mycoparasites in the soil and abiotic factors. For instance, many mycoparasites are stimulated at higher temperatures (Velvis and Jager, 1983; Jager en Velvis, 1985). Stolons sampled early in the season were only slightly colonized by mycoparasites, probably because soil temperatures are low and substrate availability is still small. Therefore the incidence of mycoparasites at the first sampling was too low to draw any conclusions on the effects of the nematicides on mycoparasitism.

All nematicides increased the incidence of *V. biguttatum* on stolons in sandy soil (Table 1). Growth of *V. biguttatum* in soil seems to be completely restricted to mycoparasitism on mycelium of *R. solani* (P.H.J.F. van den Boogert, pers. comm.). Nematicides increased the *Rhizoctonia* infection, which means that more mycelium of *R. solani* was available as a substrate for development of *V. biguttatum* on stolons and sclerotia. An additional stimulation of mycoparasitism by aldicarb, which could be

expected from the experiments in vitro on *R. solani* on PDA, was not observed in the field, where the incidence of *V. biguttatum* on stolons in aldicarb-treated plots did not differ from that in oxamyl-treated and ethoprophos-treated plots. A possible weakening of *Rhizoctonia* mycelium by aldicarb cannot, therefore, play a role in the field.

Both samplings of stolons on clay did not provide much information about any possible effects on mycoparasitism, because infection of the plants by *R. solani* or sclerotia formation on tubers was too low to offer sufficient substrate for mycoparasites to develop.

Gliocladium spp. were the only facultative mycoparasites observed on stolons. They were mostly found in the clay soil (Table 2) and only rarely in the sandy soil. Their incidence on the stolons at the second harvest was significantly stimulated by aldicarb, while ethoprophos had no effect. Jones (1976) found that among five soil fungi tested, *Gliocladium catenulatum* was the most effective one in metabolizing aldicarb. He did not mention an effect of the product on growth of the fungus. Effects on *Gliocladium* spp. might have been indirect, because mycophagous nematodes were suppressed by the nematicides. Grazing of mycophagous nematodes in the rhizosphere probably reduces the incidence of *Gliocladium* spp. on stolons. Decreased activity of mycophagous nematodes could also play a role in the increase in *V. biguttatum* in the nematicide-treated plots on the sandy soil. The role of mycophagous nematodes will be discussed in more detail in later papers.

In spring, soil temperatures are low. Therefore the activity of mycoparasites is low (Velvis, 1985). The nematicides probably did not affect the germination of sclerotia or the colonization of sclerotia by mycoparasites. This implies that inoculum density will not be influenced by nematicide application.

Germination of sclerotia is dependent on soil fungistasis. At field rates of nematicide application, fungistasis was not reduced. Therefore dormancy and hyphal growth of *R. solani* will not be influenced by the nematicides. With a bad distribution of the granulates in the field, the nematicide may accumulate at certain sites. At these sites, an increased soil fungistasis can be expected (Fig. 6). Under a high nematicide stress, excretion of antibiotics by micro-organisms may have increased (Bollen, 1979). Another explanation can be that some organisms with fungistatic properties make use of nutrients that are released by organisms killed by the nematicides.

In conclusion, the results do not show negative effects of the nematicides on the microbial antagonism to *R. solani* or on growth of *R. solani*. This makes it very likely that the nematicides greatly influence the susceptibility of the potato plant or suppress the activity of the mycophagous soil fauna. These effects will be dealt with in later papers.

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Samenvatting

De invloed van granulaire nematiciden op de groei van Rhizoctonia solani en op het microbiële antagonisme

De invloed van granulaire nematiciden op de groei van *Rhizoctonia solani* en op het microbiële antagonisme tegen deze schimmel werd onderzocht in het kader van een studie over de mechanismen die een rol spelen bij de toename van de aantasting in een met nematiciden behandeld aardappelgewas.

Ethoprofos remde de myceliumgroei van *R. solani* op aardappeldextrose agar (PDA), Czapek Dox agar (CDA) en op wateragar (WA). Aldicarb stimuleerde op PDA de groei met maximaal 14%. Op CDA en WA werd geen effect van aldicarb waargenomen. Oxamyl veroorzaakte groeiremming op CDA en WA, maar niet op PDA.

Ethoprofos en aldicarb stimuleerden de ontwikkeling van de mycoparasiet *Verticillium biguttatum* op cultures van *R. solani*. De mate van groeistimulering was afhankelijk van de voedingsbodem waarop de waard, *R. solani*, werd gekweekt. De groei van *V. biguttatum* werd sterk gestimuleerd door aldicarb en in geringere mate door ethoprofos, wanneer de waard gekweekt werd op PDA. Aldicarb had geen effect op de mycoparasiet wanneer *R. solani* op CDA gekweekt werd, terwijl ethoprofos de groei wel iets stimuleerde en oxamyl een gering remmend effect had. Op WA werd geen effect van de nematiciden op het mycoparasitisme vastgesteld.

In veldproeven op zandgrond stimuleerden de nematiciden het voorkomen van *V. biguttatum* op de stolonen. Het effect werd waarschijnlijk veroorzaakt door een verhoogde substraat beschikbaarheid (d.w.z. mycelium van *R. solani*). De verhoogde beschikbaarheid van dit mycelium kan samenhangen met een door nematiciden gereduceerde activiteit van de fungivore bodemfauna.

De bodemfungistase werd verhoogd door ethoprofos en, in geringere mate, door aldicarb bij hogere doseringen. Bij de in de praktijk aanbevolen doseringen kan echter geen effect op de fungistase verwacht worden. De toename in stengel- en stolonaantasting van aardappelen, geteeld in met granulaire nematiciden behandelde percelen, kon niet worden toegeschreven aan een direct effect van de nematiciden op de groei van *R. solani* of aan een vermindering van het microbiële antagonisme.

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