EFFECTS OF GRANULAR NEMATICIDES ON THE INFECTION OF POTATOES BY RHIZOCTONIA SOLANI

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
in het openbaar te verdedigen
op vrijdag 4 november 1988
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen

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STELLINGEN

1. Er zit meer in de grond dan er in zat.

2. De grootte van de door Rhizoctonia solani gevormde infectie-kussentjes op het ondergrondse stengeloppervlak bepaalt de grootte van de eronder gevormde lesies op de aardappelstengel.
   Dit proefschrift.

3. Afschaffing van EG-heffingen op de invoer van granen zal leiden tot een sterke toename van bodemziekten.

4. Ondanks een sterke toename van de aantasting van aardappelen door Rhizoctonia solani in met granulaire nematiciden behandelde velden geven de middelen een sterke verhoging van de knolopbrengst.
   Hofman, T.W. & s'Jacob, J.J., 1988, Gewasbescherming 19 (5): (in druk).

5. Bij de huidige teeltmogelijkheden wordt op akkerbouwbedrijven op de zand en dalgronden in het Noordoosten van het land alleen een optimaal bedrijfsresultaat verkregen door in het bouwplan een groot gedeelte aardappelen op te nemen. De teler is hierbij aangewezen op gebruikmaking van grondontsmetting en rassen met resistentie tegen het aardappelcysteaaltje.
   Mulder, A., 1988. Symposium "Bodemziekten en bodembescherming in Noordoost Nederland", Hoogeveen, the Netherlands.

6. De in een niet ontsmette akker aanwezige mycofage bodemfauna is in staat om aantasting van aardappelen door Rhizoctonia solani in grote mate te onderdrukken.
   Dit proefschrift.

7. Aldicarb en ethoprofos stimuleren de ontwikkeling van de mycoparasiet Verticillium biguttatum op Rhizoctonia solani.
Dit proefschrift.

8. De discussie over de keuze of een nulnorm of een tolerantiegrens gehanteerd moet worden voor pesticiden in het drinkwater gaat voorbij aan het gegeven dat ons voedsel vele natuurlijke en na verhitting gevormde toxische stoffen bevat. Indien deze aan dezelfde toetsen zouden worden onderworpen als welke voor pesticiden gelden, zou de consumptie van veel alledaagse levensmiddelen worden afgeraden.

9. De biologische bestrijding van bodempathogenen door introductie van antagonisten dient gepaard te gaan met maatregelen die het ecosysteem veranderen.

10. Gezien het zeer beperkte aantal doelpunten dat in de doorsnee voetbalwedstrijd gescoord wordt, is de uitslag met even veel kans op succes met dobbelstenen te voorspellen als op basis van een deskundige analyse van de elftallen.

11. Men moet medewerkers in het openbaar vervoer geen horloge geven als ze 12½ jaar in dienst zijn, maar bij indiensttreding.

12. Automatisering kost en duurt vrijwel altijd het dubbele van wat verwacht werd. Gelukkig wordt dit gecompenseerd door het feit dat het effect meestal half zo groot is als verwacht.

Proefschrift T.W. Hofman.
Effects of granular nematicides on the infection of potatoes by Rhizoctonia solani.
Wageningen, 4 november 1988.

VOORWOORD

Tijdens mijn doctoraalstudie planteziektenkunde wakkerde een leeronderzoek aan vruchtwisselingsproblemen in aardappelen mijn interesse voor bodemziekten sterk aan. Mijn begeleiders Gerrit Bollen en Klaas Scholte speelden daarbij met hun aanstekelijk enthousiasme een grote rol. De mogelijkheid om onder hun begeleiding een promotieonderzoek uit te voeren, heb ik daarom ook met beide handen aangegrepen. Van 15 november 1984 tot 1 november 1987 ben ik als wetenschappelijk assistent werkzaam geweest op een door de Landbouwuniversiteit gefinancierd project getiteld: "Effecten van granulaire nematiciden op de aantasting van aardappelen door Rhizoctonia solani".

Bij de uitvoering bleek dat het door mij gestarte onderzoek geen éénmansactie kon zijn. Met name tijdens het groeiseizoen was assistentie onontbeerlijk. Op 1 juli 1986 werd Jetty Middelkoop aangesteld als analiste om de rol van de mesofauna bij het optreden van bodempathogenen diepgaander te kunnen bestuderen. Zij vormde een zeer belangrijke schakel om het hierbij gepresenteerde onderzoek compleet te krijgen. Ik ben Jetty daarom ook zeer dankbaar voor de volhardende inzet bij de analyse van de mesofauna monsters en de assistentie bij het overige onderzoek.

Ook nematodenanalyses bleken een zeer arbeidsintensief karwei. Johan s'Jacob stond immer klaar om mij de fijne kneepjes van het determineren te leren. Bovendien ben ik hem zeer dankbaar voor de vele monsters die hij voor mij heeft geteld en de verdere onderbouwing van mijn nematologische kennis.

Voor de medewerkers van het proefbedrijf van de vakgroep Landbouwplantenteelt en Graslandkunde heb ik bewondering, omdat zij immer bereid waren om ook bij de verafgelegen proeven hulp te verlenen, onder af en toe extreme weersomstandigheden en met vaak zeer lange werkdagen.

Verder wil ik Sanderine Nonhebel, Peter Jongebloed, Meeuwi Dortmond, Han Kavelaars, Gert Sikken, Wim Bruggink, Guus Corten, Joost van den Eijnden en Fred Koops bedanken voor hun bijdragen, welke ze door middel van discussie en onderzoek als onderdeel van hun doctoraal studie onder mijn begeleiding hebben uitgevoerd.

Bij de veldproeven in Drente zijn diverse medewerkers van het H.L. Hilbrandslaboratorium voor Bodemziekten te Assen zeer behulpzaam

geweest. Ook Jan Veninga uit Hijken, op wiens land ik twee jaren veldproeven kon doen, heeft via een uitstekende proefveldverzorging, zeer bijgedragen aan het welslagen van het onderzoek.

Verder bedank ik alle studenten, medewerkers van het proefbedrijf aan de Binnenhaven en de A.P. Minderhoudhoeve en vrienden, die het mogelijk maakten om de geplande handelingen tijdens de arbeidspieken ook uitgevoerd te krijgen.

Dit proefschrift was veel minder diepgaand geweest als we geen subsidies van de Programma Commissie Onderzoek Bodembiologie hadden ontvangen. De van hen ontvangen subsidies maakten het mogelijk om ook proeven in het fabrieksaardappel-teeltgebied uit te voeren (te Hijken) en om een analiste aan te stellen om een diepgaander studie te kunnen verrichten naar de rol van de mesofauna in het optreden van bodemziekten.

Bij de verslaglegging van het onderzoek heb ik zeer veel steun ondervonden van de redactionele kwaliteiten van Gerrit Bollen. Ik heb bewondering voor de scherpheid waarmee hij mijn teksten heeft doorgespit. Ook denk ik met plezier terug aan de vruchtbare discussies tijdens het onderzoek. Klaas Scholte, Johan s'Jacob, Professor Dekker en Professor Van der Wal ben ik erkentelijk voor de becommentariëring van mijn publicaties en de adviezering bij de uitvoering van het onderzoek.

De heer Rigg, werkzaam bij de PUDOC heeft de eerste twee publicaties op het engels gecorrigeerd, terwijl Chris Kendrick de andere delen van het proefschrift heeft gecorrigeerd. Peter van Ewijk, statisticus bij Duphar B.V., heeft mij waardevolle statistische adviezen gegeven. Ook de heer Van Montfort van de vakgroep Wiskunde ben ik erkentelijk voor zijn adviezen. De heer Van de Bund, gepensioneerd medewerker van het R.I.N., heeft het zelfvertrouwen van Jetty en mij opgevoerd door de determinaties van springstaarten en mijten te controleren.

Het laatste woord van dank is gericht aan mijn huidige werkgever Duphar B.V., wegens de bereidheid mij toe te staan om gedurende een jaar aan twee verschillende banen tegelijk te werken.

Last but not least heeft Marja voor een prettige en noodzakelijke afleiding gezorgd, door me regelmatig achter mijn bureau weg te sleuren.

Al diegenen die betrokken zijn geweest bij de totstandkoming van dit proefschrift, al dan niet hier met name genoemd, wil ik daarvoor hartelijk bedanken.

CONTENTS

CHAPTER 1

INTRODUCTION

In the Netherlands the potato is one of the most profitable field crops. It is grown in most of the agricultural regions of the country, often as part of a short crop rotation, which consequently creates phytopathological risks. The more frequent a particular crop is grown in a certain field, the larger the chances are that problems will arise with soil-borne diseases. The most important soil-borne problem in the case of potato is the potato cyst nematode. Cysts of Globodera rostochiensis and G. pallida can survive for many years in the soil and when potatoes are grown in a field with a high infestation level, hatching larvae will invade roots and cause severe yield reduction.

A number of measures are taken to reduce the infestation level of the soil if potato cyst nematodes are present, or to prevent infestation of 'healthy' fields. The cultivation of potatoes on the same field is by law limited to only once in every four years. It is, however, allowed once in every three years, if use is made of varieties resistant to cyst nematodes or when the soil is chemically disinfested. If a resistant variety is grown once in every four years and chemical soil disinfestation is carried out as well, it is allowed to cultivate potatoes once every two years. In these fields, Rhizoctonia solani Kühn is one of the major soil-borne diseases.

Chemical soil disinfestation is common practice in the north-eastern part of the country where potatoes are grown for the starch industry on sandy and peat soils. In other regions, soil disinfestation is less frequently used for potatoes, but it is applied to reduce nematode problems in other field crops, e.g. sugar beet and flower bulbs. Chemical soil disinfestation is also carried out in greenhouses. Different methods of soil disinfestation are currently used. The fumigants dichloropropene and metham-sodium are applied in the autumn, prior to the year that potatoes are to be grown in the field. Both compounds are toxic to all species belonging to the soil fauna, but they are also phytotoxic and exert activity against micro-organisms (Van Berkum and Hoestra, 1979).

In the top 5-cm layer of the field, the concentrations of these fumigants are lower than in deeper layers. This means that the reduction of the number of fertile cysts will be less in the upper than in the deeper soil layers. To obtain effective control of cysts in the upper 5 cm of the field, a granular nematicide is often worked into this layer in spring, shortly before planting. Nematicides used for this purpose in the Netherlands are ethoprophos, aldicarb and oxamyl. In some soils or under certain climatic conditions it is difficult to obtain a satisfactory result by fumigation, e.g. when the soil is too moist in the autumn, temperatures are too low or the soil is too heavy. In these cases, granular nematicides may be applied shortly before planting of potatoes in order to replace the fumigant by a treatment of the upper 10 - 30 cm of the field.

In field trials with potatoes by the Department of Field Crops and Grassland Science in Wageningen, it was found that the granular nematicides ethoprophos, aldicarb and oxamyl increased the infection of stems and stolons by R. solani (Scholte, 1987). This effect was also reported by other authors (Leach and Frank, 1982; Schepers et al., 1986). However, the cause of this phenomenon has never been studied. It differed from the non-target effects of the nematicides on Verticillium dahliae, because the disease caused by this pathogen was reduced in nematicide-treated soil. This was explained by a reduced activity of parasitic nematodes, resulting in less penetration sites for the pathogen on the roots. Penetration of plants by V. dahliae is facilitated by root lesions caused by nematodes (Leach and Frank, 1982). Mechanisms that might be involved in the increased infection by R. solani in nematicide-treated soil, are effects of the nematicides on:

- the mycelial growth rate of R. solani (Chapter 2) and the development of infection structures (Chapter 3),
- the susceptibility of the host plant (Chapter 3),
- the antagonistic microflora, such as mycoparasites and the soil fungistasis (Chapter 2) and
- the mycophagous soil fauna, i.e. mycophagous nematodes (Chapters 5 and 7) or springtails and mites (Chapters 6 and 7).

In November 1984 a research project was started to investigate the mechanisms that are involved in the increased infection by R. solani in nematicide-treated soil. Special emphasis was laid on the relationship between the soil fauna and disease severity of R. solani on potato.

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CHAPTER 2

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) or to 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: aldicarb, ethoprophos, oxamyl, adsorption, mycoparasitism, side-effects, Verticillium biguttatum

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. Rodriguez-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. 10kg.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 x 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 1.

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, 5kg 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 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 ^{o}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 3 mm 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 ^{o}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 (method of 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.

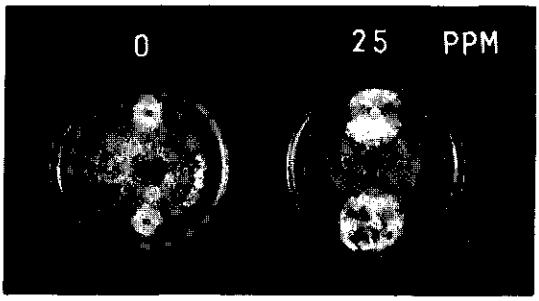

Fig. 1. Effect of aldicarb on the mycoparasitism of Verticillium biguttatum on Rhizoctonia solani.

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 x100). The most frequently found mycoparasites (V. biguttatum, Gliocladium roseum and G. catenulatum) were counted.

Fungistasis tests. To investigate whether fungistasis in the soil was affected by the nematicides, three experiments were set up.
Experiment 1. Shortly after planting of potatoes, sclerotia (c. 2 mm x 3 mm x 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 um). 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 the 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

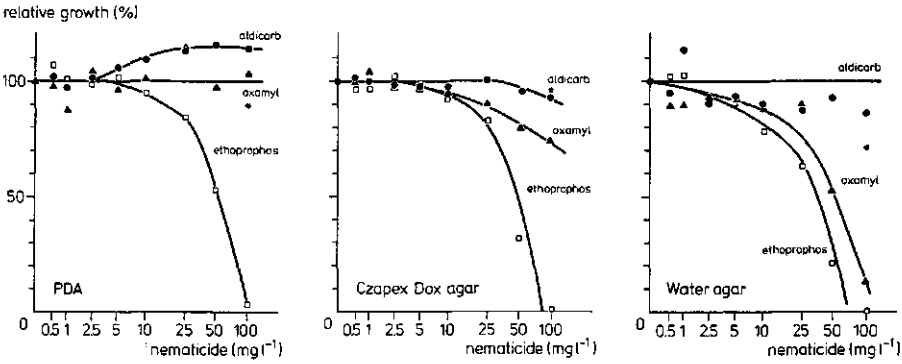

Fig. 2. Effects of nematicides on the relative radial growth of Rhizoctonia solani colonies on PDA, Czapek Dox agar or water agar. Growth is expressed as percentage of growth on agar without the nematicides. *, Growth at 0.05% ethanol, which corresponds to the concentration in the plates with the highest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

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 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

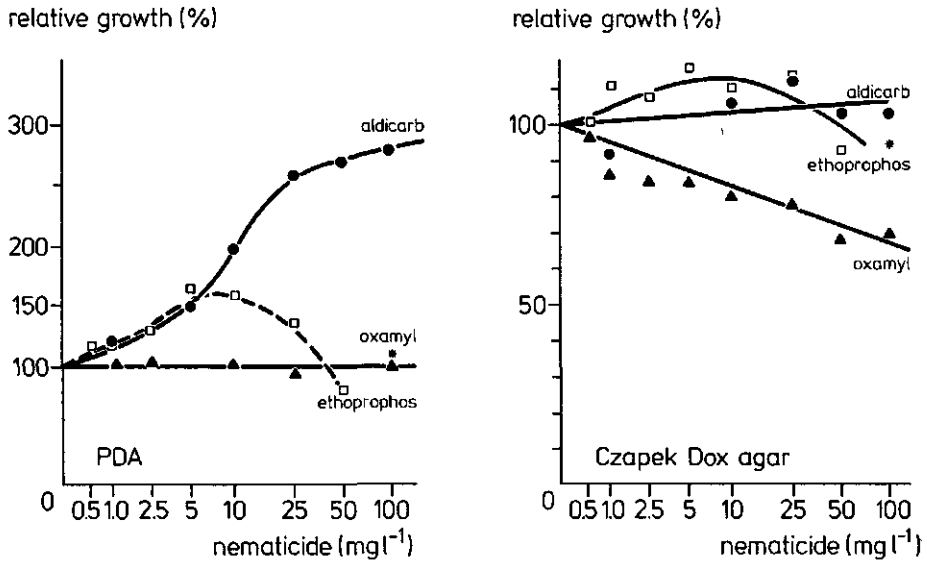

Fig. 3. Effects of nematicides on the relative radial growth of Verticillium biguttatum on Rhizoctonia solani cultures on PDA or on Czapek Dox agar. *, See Figure 2.

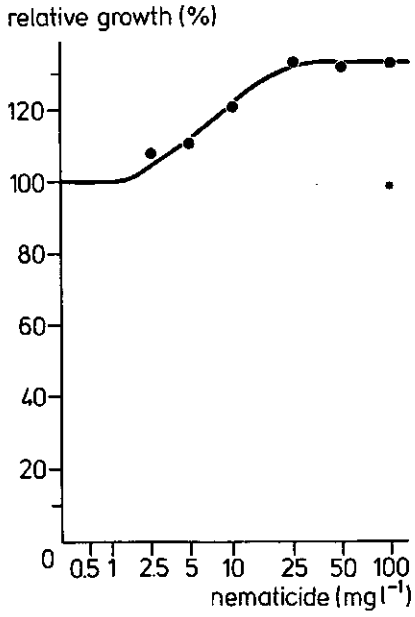

Fig. 4. Effect of aldicarb on the growth of Verticillium biguttatum on PDA.

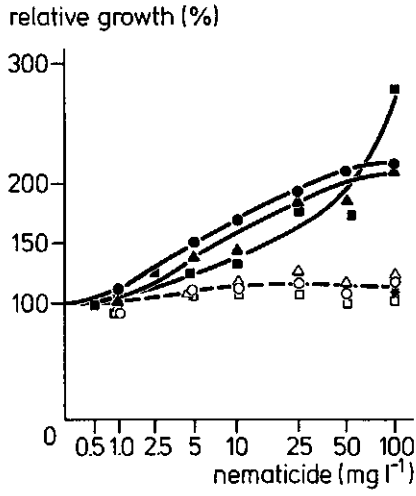

Fig. 5. Effect of aldicarb on the relative radial growth of three isolates of Verticillium biguttatum on colonies of Rhizoctonia solani on PDA (•–•–•) or Czapek Dox agar (o–o–o). Isolate Haren 17 (■,□) Gasselte 4 (▲,△) and Wildekamp (●,○).

been caused by limited development of R. solani at these concentrations (Fig 5). 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. 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%. At both dates, the nematicides had not affected the incidence of G. roseum and G. catenulatum, 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 nematicde 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). At very

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

Field	Treatment	Stolons		Sclerotia	
		17 June	30 July	13 August	25 September
F_o	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 (%) 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 = $\frac{\text{not germ. x o + class 1x1 + cl. 2x2 + cl. 3x3}}{\text{total number of sclerotia x 3}} \times 100$

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

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 soilfungistasis, because the fungistasis in unsterilized soil was not significantly higher than in autoclaved soil (Fig. 7).

The cellophane 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 soilfraction in the medium can be made with the following equation for the concentration of nematicides in soil:

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

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 medium c_l = 74 mg.l^{-1} (Fig.10)
ε_l = 1.15 l water/ 1.50 l ϱ_b = 0.85 kg/1.50 l

From these data it can be calculated 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

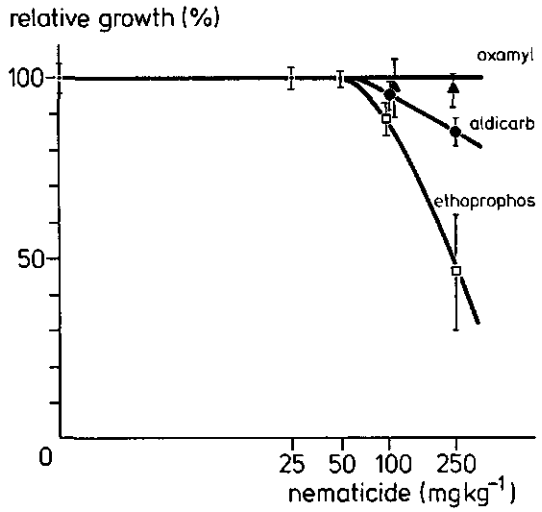

Fig. 6. Effect of nematicides on fungistasis in sandy field soil expressed by relative radial growth on cellophane. Bars represent the standard deviation.

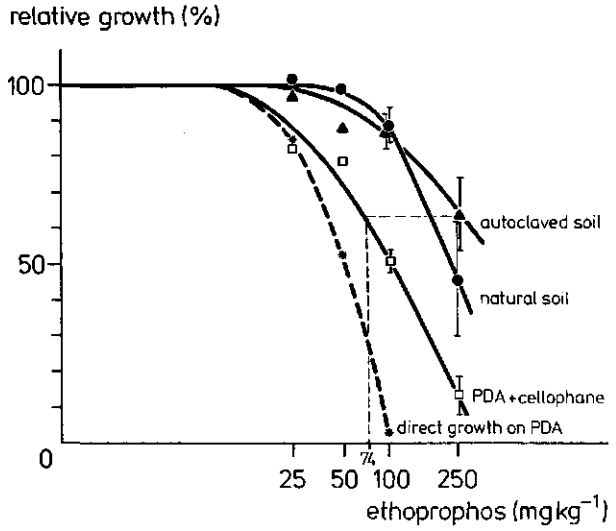

Fig. 7. Effects of ethoprophos on the relative radial growth of Rhizoctonia solani in fungistasis tests with different substrates. Bars represent the standard deviation.

upper 5 cm of soil, the content of ethoprophos is, however, 15 mg.kg^{-1} soil (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 (1974), was confirmed in our trials. Spurr 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 caried 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. 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 and 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 I). 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. The 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 the mycoparasites was low. 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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CHAPTER 3

INFECTION PROCESS OF RHIZOCTONIA SOLANI ON SOLANUM TUBEROSUM AND EFFECTS OF GRANULAR NEMATICIDES

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Abstract

The infection process of Rhizoctonia solani AG-3 was studied on potato sprouts, cv. Bintje, in growth chamber trials at 15 °C. Initially hyphae of R. solani grew predominantly in the longitudinal direction of the sprouts (runner hyphae). They tended to follow the junctions between epidermis cells as was observed by SEM. The hyphae formed side-branches mainly half-way of the subterranean parts of the sprouts. They branched several times with short swollen cells to form infection cushions. Lesions developed only underneath the infection cushions and were first observed five days after inoculation. The necrotic area was proportional to the area covered with infection cushions on the sprouts. Depth of the lesions could extend up to the vascular bundle. Sprouts were colonized only in healthy tissue in the epidermal layer underneath the infection cushion and in necrotic tissue. A few days after appearance of the lesions, R. solani formed brown, uninfective mycelium on and in the circumference of these lesions.

Aldicarb did not influence any part of the infection process. Ethoprophos delayed the emergence of sprouts but increased the number of sprouts per tuber. As soon as sprouts had emerged, growth was considerably promoted by ethoprophos. Ethoprophos delayed the appearance of lesions and reduced their size. Oxamyl showed the same effects to a smaller extent.

As the size of lesions appears to be proportional to the size of the infection cushions, any agents that change the size of the infection cushions, such as pesticides or antagonists, may alter the severity of the disease.

Additional keywords: potato, stem canker, disease severity, infection cushions, runner hyphae, ethoprophos, aldicarb, oxamyl, side-effects, non-target effect, scanning electron microscopy.

Introduction

Rhizoctonia solani Kühn infects stems, hypocotyls, roots or leaves of a wide range of host plants (Parmeter, 1970). The symptoms on potato are lesions on stems and stolons, and sclerotia on the tuber. During the vegetative period, a white collar of mycelium with basidia may be formed around the stem base. Different anastomosis groups (AG) can be distinguished in R. solani. The most common group on potato is AG-3.

Several authors (Hide and Corbett, 1974; Leach and Frank, 1982; Ruppel and Hecker, 1982; Scholte, 1987) observed in field trials an increased infection of potatoes and beet by R. solani after application of the nematicides aldicarb, oxamyl or ethoprophos. The increase in disease might be due to direct effects on the pathogen or on the host plant or to indirect effects: by a decrease of the microbial antagonism or of the activity of the mycophagous soil fauna. A previous paper showed that the enhanced incidence of the disease is not caused by an effect of the nematicides on the growth of the pathogen or on the microbial antagonism (Hofman and Bollen, 1987). This paper deals with the effects on the infection process and on the susceptibility of the host plant. The effects on the mycophagous soil fauna will be described in subsequent papers.

Although the infection process of R. solani has been studied on many hosts (Bateman, 1970; Dodman and Flentje, 1970; Fukutomi and Takada, 1979; Kenning and Hanchey, 1980; Marshall and Rush, 1980b; Matsuura, 1986), we did not find such a study on the infection of potato. Therefore, the assessment of the effect of granular nematicides was preceded by a detailed study of the various stages in the infection of sprouts and stolons of potato, with emphasis on the relation between mycelium development on the sprout surface and severity of the disease. Knowledge of this relation is important for understanding the effects of nematicides. With R. solani on rice, Marshall and Rush (1980a) found a strong correlation between size of infection cushions and lesion size. We studied whether such a correlation applies also to R. solani on potato.

By exposing sprouts to light, Van Emden (1965) induced resistance to infection by R. solani. A rapid emergence is therefore supposed to reduce the infection of the subterranean stem. Post-emergence

resistance to Rhizoctonia has also been reported in other crops, e.g. beans (Leach and Garber, 1970). Thus, stem infection of potato is reminiscent of a seedling disease. Whereas seedlings of many crops are completely killed by R. solani, lesion size on potato stems is often restricted. Probably the potato plant shows some kind of locally induced resistance in reaction to stem infection.

Pesticides can affect the resistance of the host plant (Heitefuss, 1973; Sumner, 1974; Altman and Campbell, 1977). If aldicarb, oxamyl or ethoprophos influence host resistance, they may increase the size of lesions. Because of their systemic action, aldicarb and oxamyl have the highest probability of interfering with host resistance. In the literature, reduced host resistance by aldicarb is reported only once (Tisserat et al., 1977). Aldicarb applied at three times the recommended field dosage reduced resistance of beet seedlings.

Depending on the medium used for the test, ethoprophos and oxamyl showed fungitoxicity (Hofman and Bollen, 1987). This means that application of these nematicides to sterilized soil might give a reduced or delayed infection by R. solani. In the field, ethoprophos, applied at dosages twice as high as recommended, reduced severity of root disease in cucumbers caused by R. solani and Pythium aphanidermatum (Sumner, 1978). To investigate effects of the nematicides on the interaction between potato stems and R. solani, we performed all trials in sterilized soil.

Materials and methods

Plant material and growing conditions. In all trials, plants were grown from seed tubers (25 - 35 mm in diameter) of cv. Bintje that had been stored since the August harvest at 4 °C. Before planting, sprouting was stimulated by placing the tubers at room temperature in the light for one week. During this period, they were treated against tuber-borne R. solani by dipping them in a solution of 0.9 g validamycin (Solacol) per 1. Tubers were planted in pots with autoclaved course sand and placed in a growth chamber at 15 °C and 16 h light per day. Most experiments were done from March till May.

Inoculation. R. solani was grown for 14 days at 20 °C on a perlite medium (particle diam. 1 - 5 mm). To one litre of perlite were added 500 ml distilled water, 16.7 g Czapek Dox liquid medium ingredients (Oxoid) and 5.0 g malt extract (Oxoid).

One week after planting, the inoculum was added to the sand (10 ml l^{-1}). Tubers and sprouts were taken carefully out of the sand and planted again after the soil had been inoculated.

Observation of the infection process. At various intervals after inoculation, sprouts were taken from the soil (at least 20 sprouts for each treatment). The fungus on and in the tissue was stained by immersing the sprouts in 0.1 % trypan blue in lactophenol for 15 min, rinsed twice in water and differentiated in 30 % lactic acid. The sprouts were stored in glycerol and examined for the presence and development of infection structures of R. solani.

SEM. Sprout parts were first stored in 35 % formaldehyde. Then specimens were fixed in a glutardialdehyde solution (20 g l^{-1}) in cacodylate buffer (0.1 mol l^{-1}; 24 h; pH 7.4; 20 °C). After washing twice in cacodylate buffer, specimens were fixed in OsO4 in the same buffer (10 g l^{-1}; 8 h; pH 7.4; 4 °C) and subsequently washed in buffer solution and distilled water. After dehydration in a graded series of aqueous ethanol up to pure ethanol, the specimens were critical-point-dried in liquid CO_2 and mounted on a specimen holder. Finally the specimens were sputter-coated with gold (about 15 - 20 nm thick) and examined with a Jeol 35C scanning electron microscope, operated at 15 or 25 kV.

Nematicide treatments. Before planting of seed tubers, the sand was treated with granular nematicides at dosages recommended for field application to potatoes, assuming that in the field the granulate is mixed with the upper 10 cm of soil. Ethoprophos (10 mg l^{-1}, as Mocap 20 GS), aldicarb (3 mg l^{-1}, as Temik 10 G gypsum) and oxamyl (5 mg l^{-1}, as Vydate 10 G) were mixed with the sand. To study effects at higher dosages the nematicides were also applied at double and three-fold dosages.

Post-emergence resistance. Effects of the nematicides on post-emergence resistance were tested with plants that were inoculated 23 days after planting. Before inoculation, plants were exposed to light (28 W m^{-2} from TL light sources) for about 8 days from emergence; the intensity of the light was low, which caused sprouts to become quite long. Inoculum was mixed with sand at a dosage of 30 ml l^{-1}. The mixture was placed in a layer of about 2 cm on top of the potting sand. In this trial, the effects of aldicarb and ethoprophos were tested at a dosage double the recommended dosage. The sand was irrigated as needed with Hoagland solution.

Results

Infection process. Initially, runner hyphae of R. solani grew mainly longitudinally along the sprout. The hyphae tended to follow the junctions of the epidermal cells (Fig. 1). At certain sites, mostly at half of the length of the subterranean part of the sprout, primary branches formed. They consisted of straight cells, but shorter than those of the longitudinal hyphae. From primary branches secondary branches developed, which consisted of short swollen cells. These often branched several times to form infection cushions, i.e. dense masses of swollen cells (Fig. 2). Under the infection cushions many hyphae penetrated the epidermal cells (Figs. 3 and 4). This process might be both mechanical and enzymatic. SEM showed light-colored tissue around the penetration sites (Fig. 4). Such a halo indicates that the cell walls are locally less thick (W.L. Jongebloed, personal communication). This is most likely caused by enzymatic activity.

Colonization of plant tissue was initially restricted to one or two cell layers underneath the infection cushion. Lesions developed only under infection cushions and with a margin that exceeded the infection cushion by about 2 mm. Finally, lesions were up to about 12 cell layers deep, reaching the vascular bundle of the stem. The phloem was affected and in case of a severe attack also the xylem was affected. Girdling of a sprout by this type of lesion caused death, which occurred mostly on young sprouts. Smaller lesions only slightly affected growth of stems.

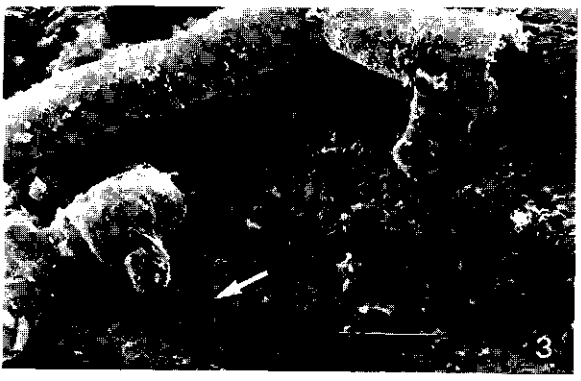

Fig. 1. Runner hyphae (and some side branches) *of* R. solani predominantly growing along the junctions of epidermal cells (three days after inoculation; bar represents 100 m).

Fig. 2. At four days after inoculation short swollen hyphae of R. solani clump together to form cushion-shaped structures (infection cushions).

Fig. 3. Penetration peg pulled away from its penetration site (arrow) at four days after inoculation (bar represents 10 m).

Fig. 4. Penetration sites in epidermal tissue under a partly removed infection cushion (bar represents 10 m). The halo around the penetration site (arrow) is characteristic for a very thin tissue layer (five days after inoculation).

R. solani colonized the dead tissue after a few days. Except during the initial colonization of epidermal cells, the mycelium was never observed in healthy tissue.

On older lesions, the mycelial mass of the infection cushions had increased, and straight-walled brown hyphae were then present on uninfected parts of the sprout. Even at this stage, colonization of plant tissue was restricted to the part directly under the infection cushion. So R. solani does not colonize sprout tissue progressively.

Contrary to earlier observations by Van Emden (1965), plants that had emerged healthy and were exposed to light were not found to be resistant to stem infection by R. solani. Even healthy- emerged sprouts can become infected so severely that they fall off.

Influence of nematicides on infection by R. solani and on sprout development. Microscopic examination of sprouts grown in sand with different dosages of aldicarb, oxamyl or ethoprophos did not demonstrate morphological changes of infection structures of R. solani. The relation between lesion size and sprout area covered with infection cushions was not affected by the nematicides.

Ethoprophos (Fig. 5) and, to a lesser extent, oxamyl delayed the rate of the infection process, possibly because of their fungitoxicity (Hofman and Bollen, 1987). Mycelial growth over the sprout surface was inhibited (unpublished results). Consequently initiation and appearance of infection cushions and appearance of lesions were also delayed and the lesions remained smaller. In untreated soil 50 % of the sprouts showed lesions 6 days after inoculation (Fig. 5). In soil with ethoprophos at 20 mg l^{-1}, this level was reached after 15 days and with oxamyl at 10 mg l^{-1} after 9 days (these dosages are double the dosages recommended for field application).

Longitudinal growth of sprouts was initially reduced, but from the emergence of the sprouts, it was promoted by ethoprophos (Fig. 6). The number of sprouts per tuber had significantly increased from 3.40 on tubers in untreated soil to 4.04 on tubers in soil with ethoprophos at 20 mg l^{-1}. Oxamyl slightly inhibited the longitudinal development of unemerged sprouts.

When sprouts were about 40 mm long in untreated soil, their apex reached the soil surface. In untreated soil, emergence was at 15 days

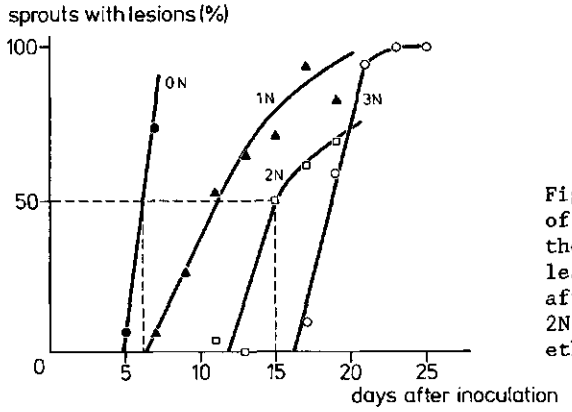

Fig. 5. Effect of three dosages of ethoprophos in the soil on the fraction of stems with lesions (%) at different times after inoculation (%). 0N, 1N, 2N and 3N: 0, 10, 20 and 30 mg ethoprophos per 1 soil.

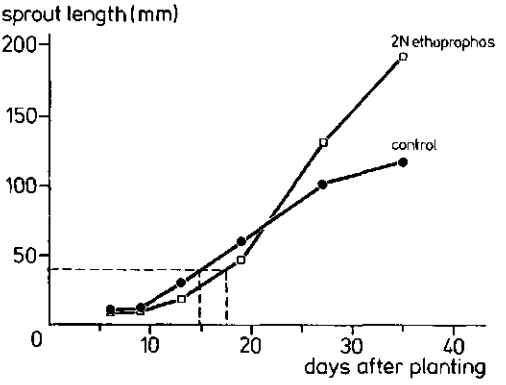

Fig. 6. Effects of ethoprophos on longitudinal growth of potato sprouts. 2N: 20 mg ethoprophos per 1 soil.

Table 1. Effect of nematicides on the fraction of potato stems girdled by lesions of R. solani, 20 days after emergence. Inoculation was at 8 days after emergence.

Treatment	Diseased stems (%)
Control	43
Aldicarb 6 mg l^{-1}	48
Ethoprophos 20 mg l^{-1}	23*
Ethoprophos 40 mg l^{-1}	17*

*significantly different from control (Wilcoxon's test; P=0.05).

after planting. Ethoprophos at 20 mg l^{-1} delayed the emergence by 3 days (Fig. 6) and the infection by 9 days (Fig 5). Oxamyl at 10 mg l^{-1} delayed the emergence by 1 day and the infection rate by 3 days.

Table 1 shows that in sterilized soil ethoprophos reduced the disease, when sprouts were inoculated after emergence. At 20 mg l^{-1}, the fraction of severely infested stems decreased from 43 % to 23 %. At

40 mg l^{-1} it was as low as 17 %. The presence of ethoprophos in the basal layer of uninoculated soil reduced infection in the upper soil layer. Therefore, there may be a systemic effect, because the upper soil layer (2 cm) was inoculated 23 days after planting but had not been treated with ethoprophos. This protection may be caused either by an increase in resistance of the sprouts or because of the presence of ethoprophos in or on the stem in the inoculated soil region.

Aldicarb did not affect the rate of the infection process by R. solani, the severity of disease or the development of sprouts.

Discussion

The size of lesions caused by infection by R. solani was proportional to the size of infection cushions on the sprout surface, this was similar as found by Marshall and Rush (1980a) on rice. The results indicate that lesions on potato sprouts are only formed after fungal penetration from infection cushions and not from lobate appressoria or simply through stomata or wounds as has been described for other hosts by Dodman and Flentje (1970). Progressive invasion of host tissue as has been reported for infected seedlings (Bateman, 1970) did not occur in potato sprouts.

Death of cells seemed to be caused by extracellular fungal enzymes or toxins, because the depth of the lesions exceeded the depth of the colonized tissue by many cell layers. Deterioration of cell walls was most prevalent in the third and fourth cell layer. Possibly, the first two layers were held together by mycelium of R. solani and deeper layers were not so seriously affected by cell wall-degrading enzymes.

The infection process was not studied on stolons, but probably proceeds in the same way as on sprouts. On roots, infection structures were never found.

The fungus can utilize nutrients released from lesions for its growth. Possibly the fungus is pathogenic as long as it grows as runner hyphae. In trials where 5 ml of perlite inoculum per litre of soil was used, stem infection rate was lower than when 0.5 ml of perlite inoculum per litre was used (Hofman, unpublished results). At the higher inoculum density the available nutrients will be used more

rapidly by the colonizing fungus than at lower inoculum densities. When lesions become older a characteristic brown mycelium appears on the sprout surface. This mycelium may represent a resting stage and may survive on plant debris in soil or on the tuber surface till another growing season. The brown mycelium develops when shortage of nutrients limits further mycelial development (Boosalis and Scharen, 1959). This mycelium did not seem to be infectious, because its side-branches never formed infection cushions. Therefore at the higher inoculum densities, the sprouts had a greater chance of escaping infection than at lower inoculum densities.

Because lesion size and size of infection cushions are related to each other, any mechanism that can influence the development of mycelium on the sprout surface (such as microbial antagonism or pesticides) may have an influence on the subsequent lesion size.

Although ethoprophos is not known to be a systemic nematicide, it caused morphological changes in potato sprouts. The number of sprouts per tuber was increased and the longitudinal development of sprouts was initially inhibited and subsequently stimulated (Fig. 6). Thus ethoprophos affects the physiology, most probably the hormonal balance, of young potato plants.

In our trials, we did not find any evidence for the existence of post-emergence resistance after exposing sprouts to light as was reported previously by Van Emden (1965). Stems that had emerged healthy, became infected when inoculated eight days after emergence, but the lesions did not girdle stems to the same extent as with pre-emergence infection. The disease-reducing activity of ethoprophos was apparent both in trials with young sprouts and with older sprouts.

It cannot be expected that nematicides increase stem infection because of phytotoxicity or stimulation of the infection process in the field. Emergence of sprouts may be delayed by ethoprophos and oxamyl. In field trials on clay, ethoprophos reduced initial development of potato plants (K. Scholte, personal communication; Hofman, unpublished results). In vitro, the relation between lesion size and surface of the infection cushions on the sprouts was not affected by any of the nematicides.

In the field, aldicarb, oxamyl and ethoprophos stimulated stem and stolon infection to the same extent (Hofman, unpublished results). The present study shows that the nematicides do not stimulate the disease by a direct effect on the interaction between the potato plant and R. solani. Ethoprophos, and to a lesser extent oxamyl, reduced the disease in sterilized soil, which was probably due to their fungitoxicity. From observations mentioned in this paper, it is expected that the increased disease severity in the field is associated with an increased amount of mycelium on the sprout surface. The cause of this increased development of mycelium on sprouts in nematicide-treated fields will be reported in later publications.

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CHAPTER 4

DISTRIBUTION AND DYNAMICS OF MYCOPHAGOUS AND MICROBIVOROUS NEMATODES IN POTATO FIELDS AND THEIR RELATIONSHIP TO SOME FOOD SOURCES

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Abstract

In three potato fields, mycophagous nematodes appeared to have similar distribution patterns and population dynamics to other microbivorous nematodes. Sampling of potato fields was carried out by three methods: using an auger, by collecting soil which was shaken from the roots (root shaking sample) and by collecting roots with the adhering rhizosphere soil (rhizosphere sample). The population densities in the rhizosphere samples were 4 to 50 times higher than those in auger samples. Samples obtained with the auger and root-shaking method yielded similar population densities. Assessment of population densities on the dying roots of potato plants after haulm killing and on flax straws, previously colonized by the fungus Rhizoctonia solani showed that numbers of the mycophagous nematodes Aphelenchus avenae, Aphelenchoides sp. and unidentified microbivorous nematodes increased several fold within a few days.

Additional keywords: sampling methods, auger sampling, Aphelenchus avenae, Aphelenchoides spp., Seinura tenuicaudata , Tylenchus s.l., Rhizoctonia solani, rhizosphere.

Introduction

Mycophagous nematodes can reduce the infection of plants by soil-borne pathogens (Schindler and Stewart, 1956; Barker, 1964; Klink and Barker, 1968; Cayrol, 1972; Jouan et al., 1972; Lemaire et al., 1972). In most of these trials, large numbers of mycophagous nematodes were introduced into the soil to reduce the disease monitored. When sampling arable land with an auger, low numbers of mycophagous nematodes are usually detected (Hofman and s' Jacob, 1988). This method of assessment did not indicate an important role of mycophagous nematodes in arable fields with respect to diseases caused by soil fungi. However, the nematicides aldicarb, ethoprophos and oxamyl increase disease severity by Rhizoctonia solani Kühn on potatoes (Scholte, 1987; Hofman and s'Jacob, 1988; Chapter 4). One of the possible explanations for this effect is that nematicides reduce the activity of the mycophagous soil fauna. For an evaluation of the role of mycophagous nematodes on disease severity, more information is needed on their occurrence within soils. This research was conducted to study their dynamics and distribution in different arable soils, where potatoes are cultivated

Since mycophagous nematodes feed on fungi, they will be most abundant in the habitats of these organisms. Fungal material occurs especially on decaying crop residues or in the rhizosphere of field crops. The traditional auger soil-sampling technique is inadequate to study the population dynamics of mycophagous nematodes, because in these samples they will only be a small fraction of the total nematode population. Therefore, alternative sampling techniques were developed, to sample soil locations, where mycophagous nematodes were expected to be most abundant.

Other microbivorous nematodes constitute the most numerous group in soil. Most of them feed on bacteria. Fungi and bacteria are decomposers of organic material. Therefore, they are most abundant at the same locations in the soil, although they may be successors to each other on a certain nutrient source. The numbers of microbivorous species without a stylet are also included in this publication. These species are referred to as microbivorous nematodes to distinguish them from the stylet-bearing mycophagous species, although mycophagous nematodes are a category of microbivorous nematodes.

Potato stems colonized by R. solani were simulated with flax straws colonized by the fungus. These baits were buried and kept in the soil for certain periods, to study the numbers of nematodes on them.

Three fields were sampled at three times throughout the growing season.

Materials and methods

Field characteristics. The three potato fields sampled were situated at different locations.

1. Hijken. The field was a sandy soil (pH-KCl 5.9, organic matter content 6 %). It had been cropped for many years in a four year rotation with potato-sugarbeet-potato-barley. The last grown crop was sugarbeet. Prior to planting, the soil was prepared with a cultivator and ploughed to approx. 20 cm deep.

   The potato cv. 'Prominent' was used. One month before planting, tubers were taken from cold storage (4 °C) and treated by dipping them in a solution of validamycin (Solacol), 0.9 g a.i. per litre, in order to kill tuber-borne inoculum of R. solani. Sprouting was activated by placing the tubers at approx. 18 °C in light for one month, before planting on 21 April, 1987.

   Four plots (6 x 10 m) were laid out in the field. Spacing of the plants was 0.75 m between the rows and 0.33 m within the rows. After ridging up six weeks after planting, the final depth of the tubers was about 15 cm.

2. Wageningen. This field was also a sandy soil (pH-KCl 6.1, organic matter content 3.3 %). The previous crops were maize (1984), cabbage (1985) and maize (1986). Prior to planting the soil was tilled with a rotating harrow.

   Six plots (6 x 10 m) were laid out and planted on 14 April 1987 with cv. 'Prominent'. Seed treatment and spacing in the field were the same as in Hijken.

3. Swifterbant. This field was a calcareous marine clay (pH-KCl of 7.2, organic matter content 4.5 %). The composition of the soil was 25 % clay, 55 % silt and 20 % sand. Potatoes had been grown in a four year rotation with potato-wheat-sugarbeet-oats. The last

cultivated crop was oats. Prior to planting the soil was tilled with an oscillating harrow.

Five plots (6 x 10 m) were laid out and planted at 23 April 1987 with cv. 'Bintje'. Seed treatment and spacing in the field were as in Hijken.

To study the build up of the nematode population in the rhizosphere of dying plants, at the end of the season, the foliage of the crop was removed by cutting, and the stubs were sprayed with dinoseb in oil (3.8 kg a.i. per ha). This was carried out in Hijken, Wageningen and Swifterbant on 31 July, 30 July and 12 August, respectively.

Sampling of nematodes. To determine the distribution of nematodes in the soil, three sampling methods were used:

1. Auger sampling. Using an auger, 40 cores were taken (20 cm deep, 2 cm diam.) from each plot. After sieving and mixing the sample, a subsample of 100 g was taken and the nematodes extracted with an Oostenbrink elutriator (Oostenbrink, 1960). The nematode suspensions were poured over a nematode filter to remove excess water and debris. The nematodes moved through the filter overnight and were collected in 100 ml tap water. Two 5 ml subsamples were taken from this suspension and analyzed under a dissection microscope (64 x magnification).
2. Root shaking. The soil was sampled by digging up potato plants and shaking off most of the adhering soil. The last adhering soil on the roots was collected. By this method soil from the rhizosphere or just outside the rhizosphere was obtained. This was carried out with 30 plants per plot. The samples were analyzed in the same manner as described under auger sampling.
3. Rhizosphere sampling. Thirty plants per plot were lifted and most of the soil was removed by shaking. A number of roots were removed from each plant, cut into pieces of approx. 2 cm long and carefully blended. A subsample of 30 g was taken, which was placed in a mistifier (Fig. 1). The samples consisted of 20 - 50 % roots on a fresh weight basis and the remainder was adhering soil. After one day the suspension was poured over a nematode filter to remove

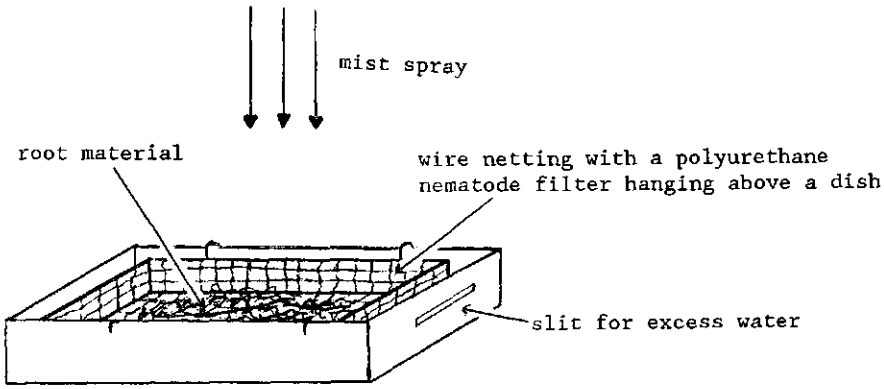

Fig. 1. A method for extracting nematodes from roots and the adhering rhizosphere soil.

debris and excess water. This was again left one day in a dish filled with 100 ml water, so that the nematodes could migrate through the filter.

Analysis of the suspensions were carried out as under auger sampling. At the first two sampling dates nematode numbers are expressed as numbers in the soil fraction of the sample, i.e. numbers in the rhizosphere soil.

At the final sampling, after the haulms had been removed, the roots were dying. As the dying roots were very light and had little adhering soil, the sample size was only 10 g. The nematode numbers at final sampling dates are expressed in numbers per 100 g sample (fresh weight of roots with adhering soil). The root fraction in these samples was often more than 90 %.

All rhizosphere samples were placed in the 'mistifier' either on the same day or one day after sampling. Auger samples and root shaking samples were stored for about three weeks at 4 °C before further processing took place.

Flax straw baits. This experiment was carried out to study the increase of mycophagous nematodes on a substrate colonized by R. solani in field soil. Pieces of flax straw (25 mm long) were washed and autoclaved in water. The excess of water was removed and the pieces

were inoculated with R. solani and incubated in flasks for eight days at 21 °C. From nylon net (pore size 2 mm), small flat parcels were made each containing ten straw pieces.

In the Hijken field, 15 parcels were buried in each of the four plots on 3 July 1987 in the middle of the ridges at an average depth of 10 cm. After 7, 16 and 28 days, five parcels were dug up from each plot. In each of the six plots at Wageningen, 12 parcels were buried on 2 July 1987. Four parcels per plot were dug up after 8, 18 and 27 days. No parcels were buried in the clay soil at Swifterbant.

After retrieval, the parcels were placed on a nematode filter in a collecting tray, filled with just enough water to soak the parcels. After incubation for one day the nematodes had passed through the filter. The suspensions were concentrated to 100 ml and analyzed as described under the auger sampling method.

Results

The year 1987 had an extremely wet growing season in the Netherlands, so in the clay soil the auger sampling could only be carried out in June, because later in the season the soil was too wet. All nematode species were most abundant on the root surface or in its immediate vicinity (Table 1). The population density of Aphelenchus avenae Bastian showed the highest ratio between numbers in rhizosphere soil and numbers in non-rhizosphere soil. At the first sampling date, numbers of A. avenae were very low. Later in the season, numbers in the rhizosphere, were higher than at the first sampling date. This was not observed with the auger samples. Extremely high numbers of A. avenae were found in the rhizosphere of dying roots.

At the first sampling date, Aphelenchoides spp. were the most abundant mycophagous nematodes in all three fields. During the season, the numbers of Aphelenchoides spp. increased in the Wageningen field, but not in the others.

Distribution of nematodes belonging to the Tylenchus s.l.-group was the least influenced by the rhizosphere. At the last sampling in the Wageningen field, their numbers increased when the roots were dying, but in the Hijken field, their numbers decreased.

Table 1. Numbers of mycophagous and microbivorous nematodes in samples taken by three methods in three potato fields.

Sampling date	Sampling method	Aphelenchus avenae	Aphelenchoïdes spp.	Tylenchus s.l.	Microbivorous species
Hijken					
1 June	auger sampling[1]	5	80	115	2775
	root shaking[1]	33	68	70	3700
	rhizosphere sampling[2]	66	205	0	19,300
31 July	auger sampling[1]	13	25	80	1490
	rhizosphere sampling[2]	196	149	131	3350
24 Aug.	rhizosphere sampling[3]	5800	180	150	7680
Wageningen					
9 June	auger sampling[1]	17	12	125	1070
	root shaking[1]	43	120	170	1620
	rhizosphere sampling[2]	0	108	238	10,300
30 July	auger sampling[1]	5	12	170	1460
	rhizosphere sampling[2]	242	259	252	5550
19 Aug.	rhizosphere sampling[3]	73,500	2080	1500	161,000
Swifterbant					
15 June	auger sampling[1]	36	44	210	846
	root shaking[1]	12	32	204	1330
	rhizosphere sampling[2]	69	562	627	21,400
12 Aug.	rhizosphere sampling[2]	1,090	170	307	5400
1 Sept.	rhizosphere sampling[3]	53,100	200	330	56,600

1 Number of nematodes per 100 g soil.
2 Number of nematodes per 100 g root adhering soil (fresh weight).
3 Numbers per 100 g roots with adhering soil (fresh weight).

The most numerous group of nematodes were the microbivores (Table 1). After removal of the foliage and spraying the stubs with dinoseb in oil, a strong increase in population density of A. avenae and microbivorous nematodes occurred in all fields. In the fields at Hijken and Swifterbant, the number of A. avenae was about equal to the numbers of microbivorous nematodes at the final sampling. In the Wageningen field, the population density of microbivorous nematodes was twice the density of A. avenae. Nematode numbers did not differ much in soil samples obtained with an auger or by root shaking, the root shaking method was omitted at the later sampling dates.

Flax straw baits. The most abundant mycophagous nematode species extracted from the baits were A. avenae and Aphelenchoides spp. (Figs. 2 and 3). Microbivorous nematodes were much more abundant than any other species. Tylenchus spp. and Seinura tenuicaudata were also extracted from the straws. At the second sampling date higher numbers of nematodes were extracted from the straws. From straws that had been buried for 27 or 28 days much lower numbers were extracted than from previous samples. The number of nematodes extracted from straws lifted from the Wageningen field was higher than those from the Hijken field at most sampling dates.

Discussion

The nematode numbers clearly show that sampling of the soil with an auger is unsuitable for a precise study of mycophagous populations in field soil (Table 1). The fraction of mycophagous nematodes in samples taken by auger was on average 1 %, so that extremely large subsamples need to be counted for a precise estimation of their numbers in the soil.

The method of sampling may have influenced the number of nematodes detected. On 15 June, when auger samples were taken on clay, the soil had a high moisture content. The auger compressed the soil and this may have injured nematodes. On wet sandy soil, the auger appeared to compress the soil less. Another factor that may have reduced nematode numbers was storage of the auger samples for about three weeks at 4 °C, before extraction was carried out. The rhizosphere samples were placed in the 'mistifier' on the sampling day or one day later. In rhizosphere samples, nematode numbers were probably not influenced by compression of the sample or by long storage under unfavourable conditions.

Mycophagous nematodes were abundant in the rhizosphere of the potato plant. It is expected that mycophagous and other microbivorous nematodes migrate to and reproduce at locations in the soil where food is available in large quantities. A relative abundance of fungi and bacteria is expected to be found on decaying organic material and in the rhizosphere where root exudates, dying root hairs and root cells supply nutrients for the microflora. The thickness of the rhizosphere

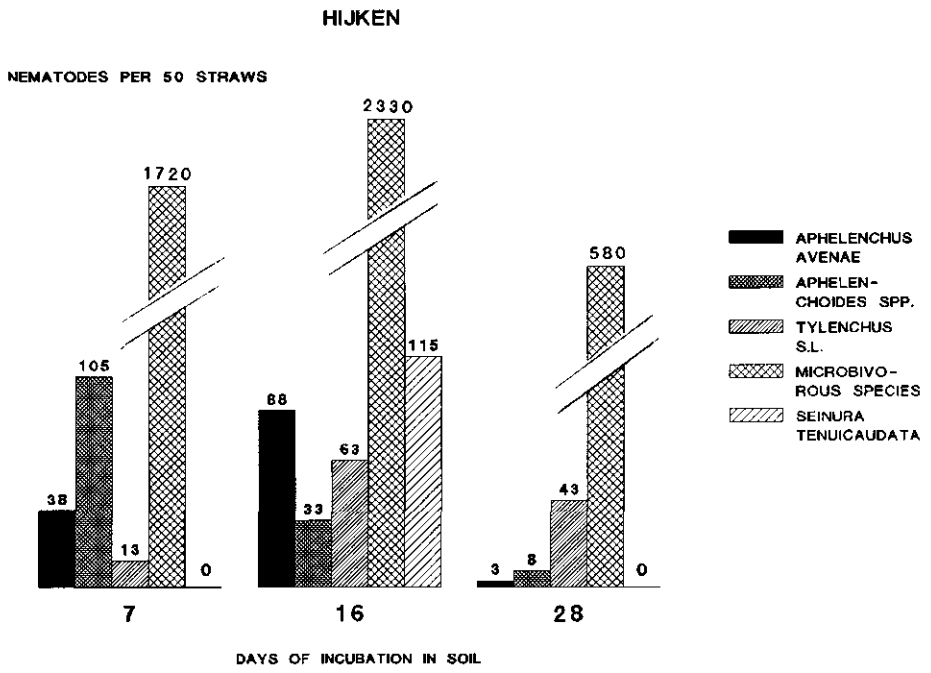

Fig. 2. Nematode populations extracted from flax straws, incubated for different periods in a potato field at Hijken.

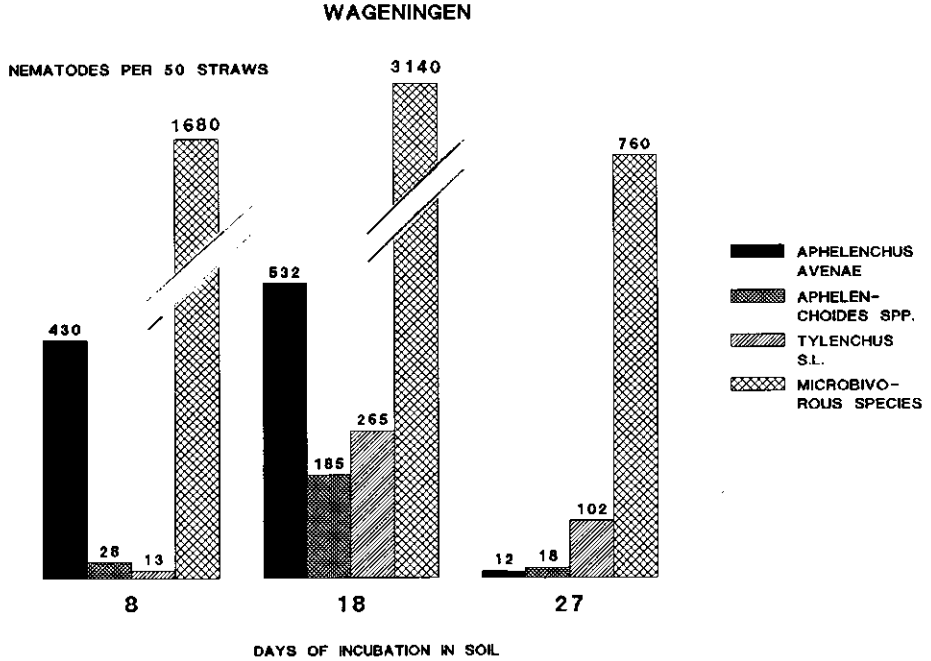

Fig. 3. Nematode populations extracted from flax straws, incubated for different periods in a potato field at Wageningen.

zone depends on the length of root hairs and on the quantity and diffusion gradient of exudates in the soil.

Towards the end of the season more potato roots die in the ridge, which is where the samples were taken. This means that an increasing amount of fresh organic material becomes available for the microflora and consequently for the mycophagous and other microbivorous nematodes feeding on the microflora.

The microbial activity makes it possible that rhizosphere soil adheres more to the roots than non-rhizosphere soil. The samples obtained with the root-shaking method, therefore, may have mainly contained soil derived from the soil layer outside the rhizosphere. This could explain why nematode numbers were almost equal to those of the auger samples.

At the first sampling date, numbers of Aphelenchoides spp. were higher than A. avenae in both rhizosphere and non-rhizosphere soil. At lower temperatures, Aphelenchoides species have a higher reproduction rate than A. avenae (Cayrol, 1967; Dao, 1970; Rössner and Nagel, 1984). During the growing season their numbers were almost equal in the two fields on sandy soil. After the foliage was removed, the increase in numbers of all nematode species was more rapid in the field at Wageningen than in that at Hijken. This difference could be due to differences in soil fertility, organic matter content, soil moisture and soil temperature. Besides a difference in organic matter content of the soil, the average temperature was approx. 2 °C lower in Hijken than in Wageningen, and the soil was also wetter, because of its smaller drainage capacity.

After seven days incubation in the field, large numbers of mycophagous and microbivorous nematodes had colonized the flax straw baits (Figs. 2 and 3). The presence of high numbers of nematodes feeding with bacteria was probably due to a rapid increase of bacteria on the flax straws in the soil. However, it cannot be concluded that the numbers observed were the maximum population densities, since it is possible that A. avenae, Aphelenchoides spp. or other microbivorous nematodes had reached their maximum between the two sampling dates.

An almost daily heavy rainfall during the second half of July, may have reduced the nematode populations. This can be seen from the

numbers observed at the three sampling dates in the trial with flax straw baits (Fig. 2) and from the lower numbers of microbivorous nematodes in the rhizosphere samplings at the second sampling date. Of course nematode numbers on the straws also may have decreased because of an exhausted nutrient source.

At the second sampling date of flax straw baits at Hijken large numbers of Seinura tenuicaudata were found. Hechler (1963) found that this species was predatory and could be reared on cultures of A. avenae. Other nematode species probably also are a prey for S. tenuicaudata, which explains its sudden appearance at the second sampling. The absence of S. tenuicaudata at the last sampling date is, therefore, explained by the drop in nematode numbers, resulting in a shortage of food for the predator.

Species of Tylenchus s.l. were found in most samples. Their food preference is not well understood, some species may be mycophagous. Their small size and slow reproduction rate mean that it is not expected to influence the incidence of soil-borne diseases. Attempts to rear Tylenchus species or S. tenuicaudata on cultures of the fungi R. solani or Alternaria porri were unsuccessful.

Our trials demonstrate, that a very rapid increase of A. avenae, Aphelenchoides sp. and other microbivorous nematodes occurs when food sources become available. Mycophagous nematodes increased to high numbers within one week after introduction of a suitable substrate. Large numbers of mycophagous nematodes were only detected in the rhizosphere and not in the samples collected with an auger.

A subsequent paper will present data on the numbers of mycophagous nematodes, that are required to reduce incidence of Rhizoctonia solani.

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CHAPTER 5

STIMULATION OF RHIZOCTONIA SOLANI ON POTATO BY NEMATICIDES AND THEIR EFFECT ON MYCOPHAGOUS AND MICROBIVOROUS NEMATODES

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Abstract

The granular nematicides aldicarb, ethoprophos and oxamyl increased stem infection and black scurf on potatoes by Rhizoctonia solani in three fields with different cropping histories and soil characteristics. Similarly, stem infection increased in a field where the insecticide lindane was applied. The infection by R. solani was only slightly afffected by these nematicides in a field treated with the fumigant dichloropropene.

Aldicarb, ethoprophos and oxamyl reduced the populations of Aphelenchus avenae, Aphelenchoides spp. and other microbivorous nematodes. Numbers of A. avenae in the rhizosphere of soil treated with granular nematicides were related to the incidence of black scurf on tubers.

In untreated field soil, baits of flax straws colonized by R. solani were colonized by A. avenae and Aphelenchoides spp. within one week. These species were rarely observed in aldicarb-treated soil.

Lindane did not affect the population of nematodes, but its application was also followed by an increased stem infection and black scurf incidence. It is suggested that soil arthropods also play an important role in the suppression of R. solani in arable fields.

Additional keywords: Aphelenchus avenae, Aphelenchoides spp., nematode sampling methods, aldicarb, oxamyl, ethoprophos, lindane, dichloropropene, disease severity

Introduction

Application of nematicides is common practice in arable fields to minimize yield reduction resulting from parasitic nematodes and root-feeding insects. However, the products not only suppress the soil fauna parasitic to plants, but also the majority of other soil-inhabiting animals. Several authors have reported an increased disease severity by Rhizoctonia solani Kühn on various crops after application of aldicarb, ethoprophos or oxamyl (Sumner, 1974 and 1978; Leach and Frank, 1982; Ruppel and Hecker, 1982; Schepers et al., 1986; Scholte, 1987; Hofman and s'Jacob, 1988).

As part of a study on the mechanisms involved in the increased infection, it was shown that the nematicides did not stimulate the mycelial development of R. solani or reduce its microbial antagonism (Hofman and Bollen, 1987). In addition they did not cause a direct stimulation of the infection process (Hofman and Jongebloed, 1988). Therefore, other mechanisms were more likely to explain the increased infection of potatoes in nematicide-treated soil.

This paper reports the effects of nematicides on the disease severity of R. solani on potatoes in three fields with different cropping histories and soil types. The effects of dichloropropene, a broad spectrum soil fumigant, and lindane, an insecticide/acaricide, were studied to learn more about the role of mycophagous nematodes in the reduction of R. solani. Their numbers were assessed in nematicide-treated and in untreated plots, using special sampling methods (Chapter 4).

Some mycophagous nematode species originating from field soil can reproduce very rapidly under favourable conditions (Townshend, 1964; Pillai and Taylor, 1967; Perper and Petriello, 1977; Hofman and s'Jacob, 1988). During the growing season, food (i.e. fungal mycelium) is mainly available for mycophagous nematodes in the rhizosphere and on crop residues in the soil. R. solani is most prevalent on the underground plant parts. On stems and stolons, the fungus forms infection cushions, i.e. dense masses of mycelium from which infection takes place (Hofman and Jongebloed, 1988). The mycophagous soil fauna may reduce the formation and size of infection cushions. Therefore, application of a nematicide that reduces the activities of the

mycophagous fauna may result in larger and more lesions on stems and stolons caused by R. solani.

It is common practice in seed potato production in the Netherlands to destroy the foliage at the end of July in order to prevent virus infection of the seed. However, haulm destruction induces R. solani to form sclerotia (black scurf) on tubers (Spencer and Fox, 1979; Dijst, 1986). It is likely that incidence of black scurf is related to the amount of vital mycelium that is formed on the underground plant parts during the growing season. Nematode populations were also assessed at the moment that the seed tubers were harvested.

The best locations in the soil to study nematicidal effects would have been where R. solani infects the potato plants, i.e. subterranean stems and stolons. However, this is practically impossible, since when lifting a potato plant the stems and stolons are almost free of soil. If any nematodes are present on the surface of stems and stolons, many are lost during transport to the laboratory. The main goal of the research was to study the effects of the nematicides on mycophagous nematodes. Although the nematode population may be different between rhizosphere soil and soil immediately surrounding stems and stolons, the effects of nematicides are probably comparable at both sites.

Fungi and bacteria are most abundant at locations in the soil where there are abundant nutrients (in the rhizosphere and on crop residues). In an arable field during the growing season most fungi and bacteria are found in the rhizosphere. They may be successors of each other or be present at the same time at the same locations. Therefore, both mycophagous nematodes and nematodes that feed on bacteria should be most abundant in the rhizosphere. Since the majority of the nematode population in the rhizosphere are bacterivorous species, the effects of the nematicides on these species are also discussed for comparison.

Aphelenchus avenae Bastian and Aphelenchoides spp. Fischer are referred to as mycophagous nematodes. Species without a stylet that may feed on bacteria, amoebae, algae or fungi are referred to as microbivorous nematodes, although this name could also mean that they are stylet-bearing mycophagous species.

The influence of mycophagous nematodes on disease severity in inoculation trials will be reported in a later paper (Chapter 7). The effects of nematicides on the mycophagous mesofauna (Acari and Collembola) and the effects that mesofauna species have on disease severity, will also be reported in later papers (Chapters 6 and 7).

Materials and methods

Three fields at different locations were used to study the effects of granular nematicides, dichloropropene and lindane on stem infection and black scurf and on mycophagous nematodes. Disease severity by R. solani was assessed by rating stem infection and black scurf. Nematode numbers were studied by analyzing samples from the soil, the rhizosphere and baits.

Field characteristics. The experimental fields were situated at three locations;

1. Hijken. This field was a sandy soil (pH-KCl 5.9, organic matter content 6 %). The field had been cropped for many years in a four year rotation with potato-sugarbeet-potato-barley. The last crop before the potato crop of this experiment, was sugarbeet. Prior to planting the soil was prepared with a cultivator and ploughed to approx. 20 cm deep.

   The potato cv. 'Prominent' was used. One month before planting, tubers were taken from cold storage (4 °C) and treated by dipping in a solution of validamycin (Solacol), 0.9 g a.i. per litre, in order to kill tuber-borne inoculum of R. solani. Sprouting was activated by placing the tubers at approx. 18 °C in light for one month, before planting on 21 April, 1987.

   The experimental field had three subfields. Two of these were each divided into 16 plots. The plots (6 x 10 m) were treated with ethoprophos (10 kg a.i. per ha, as Mocap 20 GS), aldicarb (3 kg a.i. per ha, as Temik 10 G), oxamyl (5 kg a. i. per ha, as Vydate 10 G) or remained untreated. One of the two subfields had previously been treated with dichloropropene (240 l ha $^{-1}$ Telone II) on 3 Nov. 1986.

In the third subfield the effects of dichloropropene were studied by treating four plots on 3 Nov. 1986 and leaving four plots untreated.

Each subfield was laid in a randomized complete block design with four replicates. Inoculum of R. solani and granular nematicides were applied at the planting day and were worked into the soil to a depth of approx. 10 cm with a rotating harrow. Spacing of the plants was 0.75 m between the rows and 0.33 cm within the rows. After ridging-up at six weeks after planting, the final depth of the tubers was about 15 cm.

2. Wageningen. This field was also a sandy soil (pH-KCl 6.1, organic matter content 3.3 %). The previous crops were maize (1984), cabbage (1985) and maize (1986). Prior to planting the soil was tilled with a rotating harrow.

In this trial the effects of lindane (1.47 kg a.i. per ha, as Chimac-Lindaan) and aldicarb (3 kg a.i. per ha, as Temik 10 G) were studied in a randomized complete block design with six replicates, and a plot size of 6 x 10 m. Lindane, aldicarb and inoculum of R. solani were applied at the planting date, 14 April, 1987, and worked into the soil with a rotating harrow till a depth of approx. 10 cm. The cv. 'Prominent' was used and seed treatment and spacing were as in Hijken.

3. Swifterbant. This field was a calcareous marine clay (pH-KCl of 7.2, 4.5 % organic matter). The composition of the soil was 25 % clay, 55 % silt and 20 % sand. Potatoes had been grown in a four year rotation with potato-wheat-sugarbeet-oats. The last cultivated crop was oats. Prior to planting the soil was tilled with an oscillating harrow.

Plots (6 x 10 m) were treated with 3 kg a.i. per ha aldicarb (as Temik 10 G on corncob), 9 kg a.i. per ha aldicarb, 10 kg a.i. per ha ethoprophos (as Mocap 20 GS), 30 kg a.i. per ha ethoprophos or remained untreated. The experimental design was a randomized complete block design with five replicates. Nematicides and inoculum of R. solani were worked into the soil with an oscillating harrow to a depth of approx. 6 cm on the planting date, 23 April, 1987. The cv. 'Bintje' was used and seed treatment and spacing in the field were as in Hijken.

Inoculation of experimental fields with R. solani. To obtain an evenly distributed infection with R. solani in the field, all the experimental fields were inoculated. Inoculum was prepared on a perlite substrate. Five hundred ml Czapek Dox liquid medium and 5 g malt extract (Oxoid) were added to one litre perlite (particle size between 1 and 5 mm). The autoclaved substrate was inoculated with a pathogenic strain of R. solani. The cultures used were 19 days old for the field at Wageningen and 26 days old for the field at Hijken. The inoculum was spread over the field at a dosage of 1.67 ml m^{-2}. At the Swifterbant trial the culture was 28 days old and was applied at a dosage of 6.67 ml m^{-2}.

Assessment of disease severity of R. solani. Stems were assessed for stem infection by R. solani on two separate days and tubers were assessed for black scurf incidence at a third date.

Stem infection. Thirty plants were lifted in each plot in June and in July or August. Disease severity was determined by classifying each plant according to the scale presented in Table 1. Small lesions may erroneously be attributed to infection by R. solani, although caused by

Table 1. Disease rating of stems and tubers for infection by Rhizoctonia solani.

Class	Stem infection	Black scurf
0	no lesions	no sclerotia
1	few very small lesions	maximally three small sclerotia per tuber
2	light infection: no more than 25 % of the stems show a nearly closed lesion around the stem	tuber lightly covered by sclerotia
3	moderate infection: 25 - 50 % of stems show a nearly closed lesion around the stem	tuber moderately or heavily covered by sclerotia
4	severe infection: > 50 % of stems show a (nearly) closed lesion around the stem	------
5	very severe infection: all stems are killed	------

other factors. Disease severity is, therefore,best expressed as the percentage of plants with definite symptoms caused by this pathogen, i.e. disease classes three, four and five. Plants in class four were by far the most common.

Black scurf. At the second sampling date, the foliage of 30 plants per plot was cut off and the stubs were killed by spraying with dinoseb in oil (3.8 kg a.i. per ha). This treatment induces R. solani to form black scurf on tubers, this becoming apparent at least two weeks later (Spencer and Fox, 1979; Dijst, 1987). Three weeks after haulm destruction, three tubers were randomly taken from each plant and assessed according to the classes in Table 1. Due to circumstances, in Hijken the haulm destruction in the third subfield was carried out seven days later than in the other two subfields. However, sampling of all tubers was carried out on the same day leading to a lower incidence of black scurf in the third subfield. The incidence of black scurf is expressed as the percentage of tubers in classes 2 and 3. Tubers with sclerotia most frequently belonged to class 3.

Sampling of nematodes. Nematode populations were determined by two methods. See Chapter 4 for more details.

1. Auger sampling. Using an auger, 40 cores (20 cm long, 2 cm diam.) were taken per plot for a qualitative and quantitative assessment of the population of nematodes.
2. Rhizosphere sampling. Thirty plants per plot were lifted and most of the soil was removed from the roots by carefully shaking. From each plant a number of roots were sampled. Subsamples of 30 g were analyzed for nematodes. The soil fractions in these samples were also assessed. At the first two samplings the number of nematodes were calculated per 100 g rhizosphere soil. At the third sampling, when roots were dying after removal of the foliage and very little soil adhered to them, the number of nematodes was calculated per 100 g root sample, including the adhering soil.

   In Swifterbant, no auger samples were taken at the second and third sampling dates, because the soil was too wet for sampling. In Wageningen, no rhizosphere samples were taken at the second sampling date.

Table 2. Effects of granular nematicides and dichloropropene on infection of potato plants by Rhizoctonia solani and incidence of black scurf on tubers in three fields at Hijken.

Field	Treatment	Incidence of plants with at least a moderate infection (%) 1 June	31 July	Incidence of tubers with black scurf (%) 24 August
non-fumigated	untreated	11	12	29
	ethoprophos	23*	47*	33
	aldicarb	14	30*	45*
	oxamyl	6	24	33
fumigated with dichloro-propene	untreated	9	10	28
	ethoprophos	5	14	24
	aldicarb	14	18	34
	oxamyl	7	9	31
		1 June	7 August	24 August[2]
dichloro-propene versus untreated	untreated	0.9	13	7
	fumigated	6.2	13	13
		p=0.063[1]	-	-

* Significantly different from untreated with Dunnett's procedure ($p \leq 0.05$).
[1] Probability of a significant difference with two-sample t-test.
[2] Harvested 17 days after removal of foliage instead of 24 days.

Table 3. Effects of ethoprophos and aldicarb on infection of potato plants by R. solani and incidence of black scurf on tubers in a field at Swifterbant.

Treatment	Incidence of plants with at least a moderate infection (%) 15 June	12 August	Incidence of tubers with black scurf (%) 1 September
untreated	2	23	16
ethoprophos 10 kg ha^{-1}	2	16	19
ethoprophos 30 kg ha^{-1}	1	16	27
aldicarb 3 kg ha^{-1}	5	24	25
aldicarb 9 kg ha^{-1}	3	38*	38*

* Significantly different from untreated with Dunnett's procedure ($p \leq 0.05$).

Table 4. Effects of aldicarb and lindane on infection of potato plants by R. solani and incidence of black scurf on tubers in the field at Wageningen.

Treatment	Incidence of plants with at least a moderate infection (%) 9 June	30 July	Incidence of tubers with black scurf (%) 19 August
untreated	6	13	8
lindane	10	38*	46*
aldicarb	12	49*	55*

* Significantly different from untreated with Dunnett's procedure ($p \leq 0.05$).

Flax straw baits. This trial was carried out to study effects of pesticides on the population of mycophagous nematodes in field soil on a substrate colonized by R. solani (Chapter 4).

At Hijken, 15 parcels were buried per plot in the middle of the ridges at a depth of 10 cm on 3 July 1987. Each parcel contained ten straws of 25 mm, which had previously been colonized by R. solani during incubation under sterile conditions. They were buried in the non-fumigated subfield in untreated, aldicarb- and ethoprophos-treated plots. After 7, 16 and 28 days, five parcels were dug up from each plot. At Wageningen 12 parcels per plot were buried in untreated, lindane- and aldicarb-treated plots, on 2 July 1987. Four parcels per plot were dug up after 8, 18 and 27 days.

After retrieval, the parcels were placed on a nematode filter in a collecting tray, filled with just enough water to soak the parcels. After incubation for one day the nematodes had passed through the filter. The suspensions were concentrated to 100 ml and analyzed as with the other nematode samples.

Statistical analysis. All data were analyzed using an analysis of variance. Prior to analysis, percentages and nematode numbers were transformed by 2arcsin-square root and square root transformations, respectively. The effects were further analyzed by comparing the means from the treated and untreated plots with Dunnett's-test.

Results

Effects of the pesticides on stem infection and black scurf. Aldicarb, ethoprophos and lindane increased both stem infection and black scurf of potatoes in three fields (Tables 2 - 4). However, the effects of nematicides were different at each location. At Hijken in the non-fumigated subfield, aldicarb and ethoprophos stimulated R. solani more than oxamyl (Table 2). In oxamyl-treated plots, an increased stem infection was only observed at the second harvest, but the effect was not significant. In the fumigated subfield, the effects of the nematicides on R. solani were only very small. The fumigant, dichloropropene, only slightly affected the disease. At the first

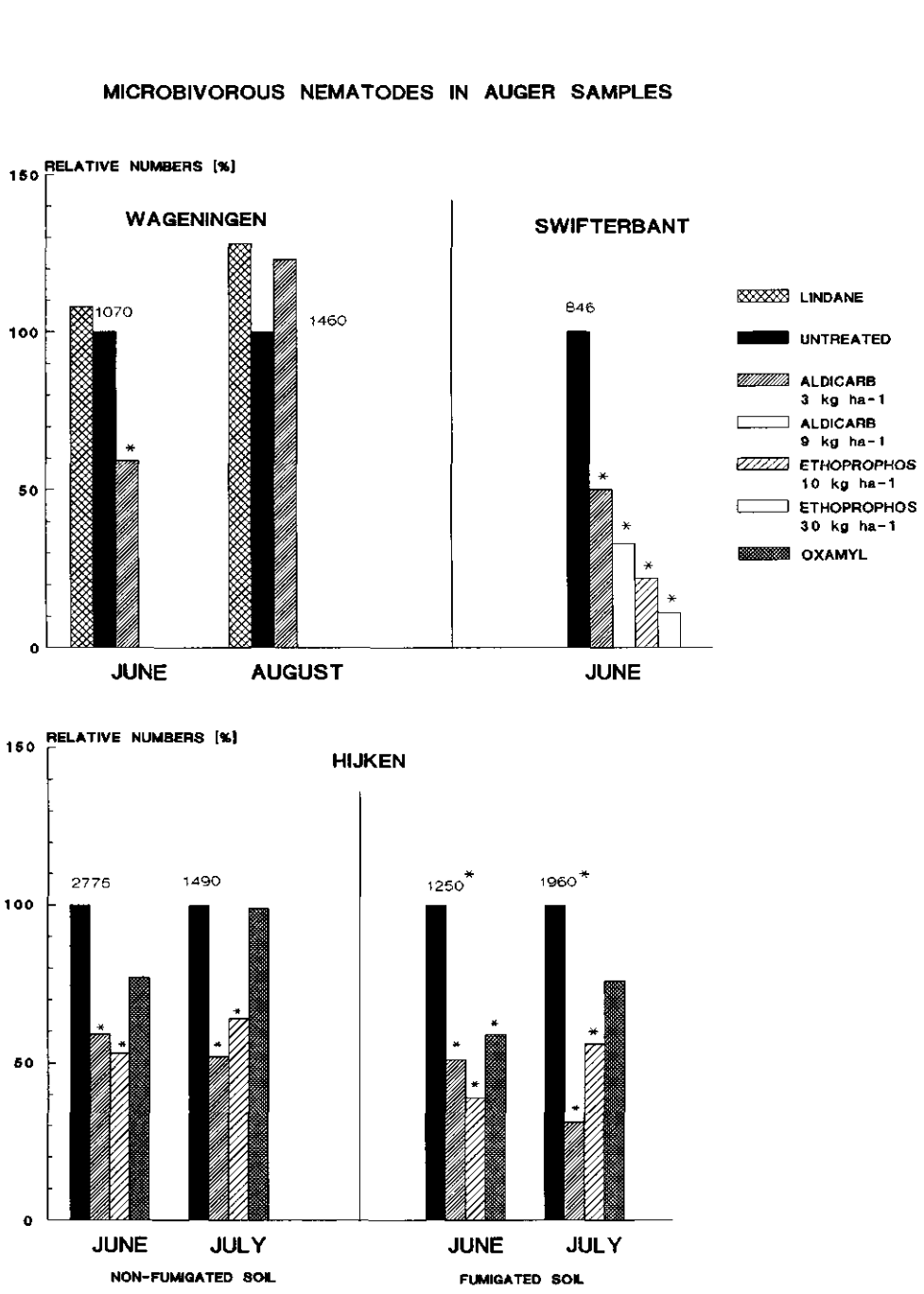

Fig. 1. Relative numbers of microbivorous nematodes in different field soils treated with granular nematicides, lindane or the fumigant dichloropropene. Samples were taken by auger. results are expressed as a percentage of the untreated controls. Numbers indicate the number of nematodes per 100 g in untreated soil. An asterisk indicates a significant difference between numbers in untreated and treated plots (also with respect to fumigated versus non-fumigated plots).

harvest, the infection of stems in fumigated plots was more severe than in non-fumigated plots, but this difference was not observed later in the season.

In Swifterbant, in plots treated with aldicarb at the recommended dosage, R. solani was slightly increased, but when aldicarb was applied at 9 kg a.i. per ha, stem infection and black scurf were strongly increased (Table 3). In plots treated with 30 kg ethoprophos per ha, the stem infection was slightly reduced and black scurf was slightly increased. In plots with the recommended dosage of ethoprophos (10 kg a.i. per ha) a somewhat reduced stem infection at the second sampling date was observed, but there was no effect on black scurf.

In Wageningen, aldicarb, and to a smaller extent lindane, strongly increased both stem infection and black scurf (Table 4).

In all fields, the effects of the pesticides on stem infection were more apparent at the second sampling than at the first one. In untreated plots in the sandy fields, the disease severity had hardly increased between the two samplings, whereas disease severity was much increased in the nematicide-treated plots.

Effects of nematicides on mycophagous and microbivorous nematodes A previous study has shown, that mycophagous and microbivorous nematodes are more abundant in rhizosphere soil, than in soil randomly sampled with an auger (Chapter 4). The mycophagous species recorded were Aphelenchus avenae and Aphelenchoides spp.. These nematodes can be reared on agar plates covered with the fungi Alternaria porri or R. solani. In the soil sampled by auger, the numbers of mycophagous nematodes were very low. The effects of the nematicides could, therefore, not be properly studied with auger samples. Numbers of microbivorous nematodes were much higher than mycophagous species in the auger samples, so it was possible to analyze the effects of nematicides on their numbers in auger samples.

At the first sampling date at Hijken, microbivorous and mycophagous nematode populations were lower in fumigated plots than in non-fumigated plots. At the later sampling dates, it was the other way round, all numbers being higher in plots that had been fumigated (Figs. 1 - 4).

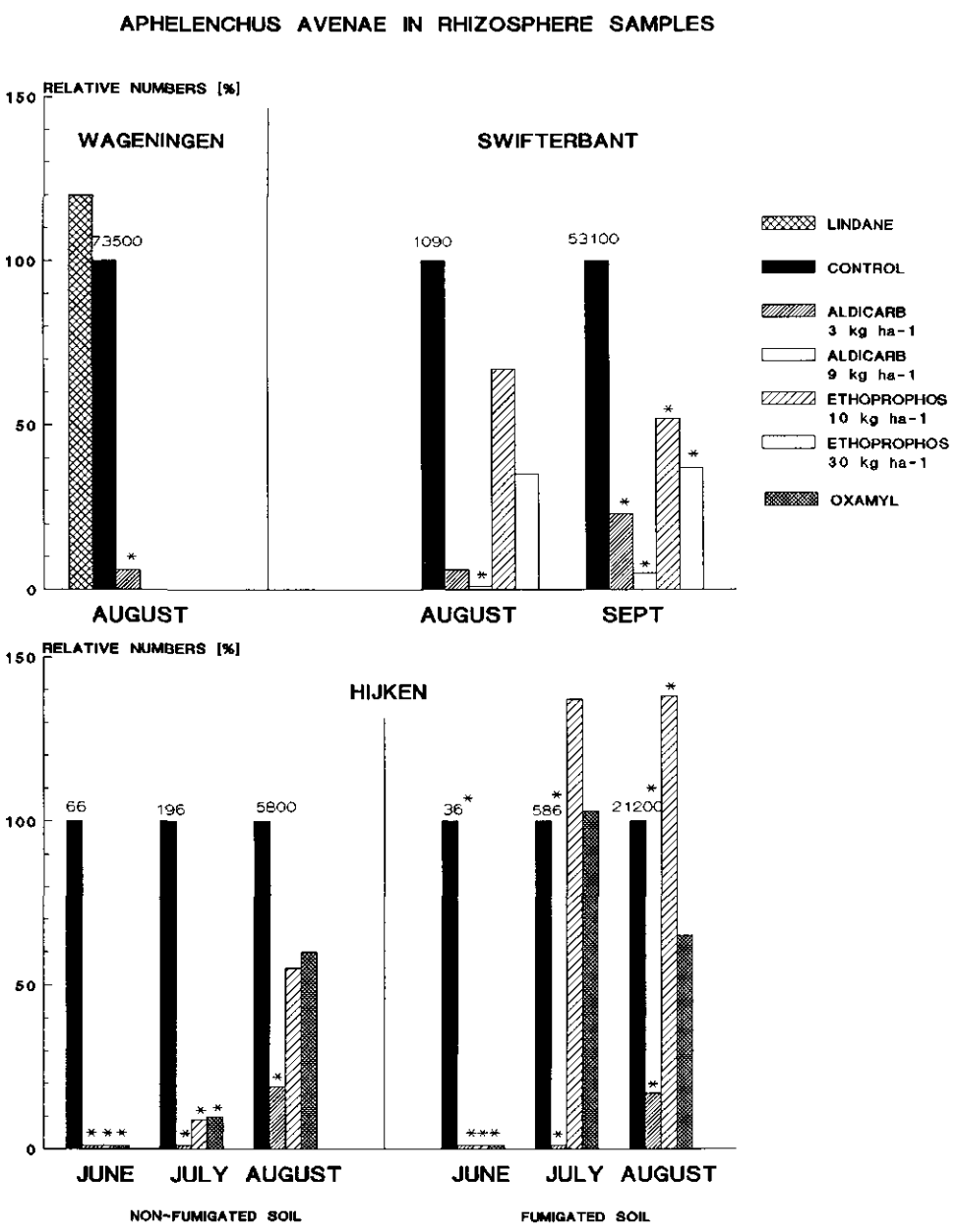

Fig. 2. Population densities of Aphelenchus avenae in rhizosphere soil from plots treated with granular nematicides, lindane or the fumigant dichloropropene. Results are expressed as a percentage of numbers in untreated plots. Numbers indicate the number of nematodes per 100 g in untreated rhizosphere soil, except the August and September samplings, where numbers are indicated per 100 g dying roots including the adhering soil. An asterisk indicates a significant difference between numbers in untreated and treated plots (also with respect to fumigated versus non-fumigated plots).

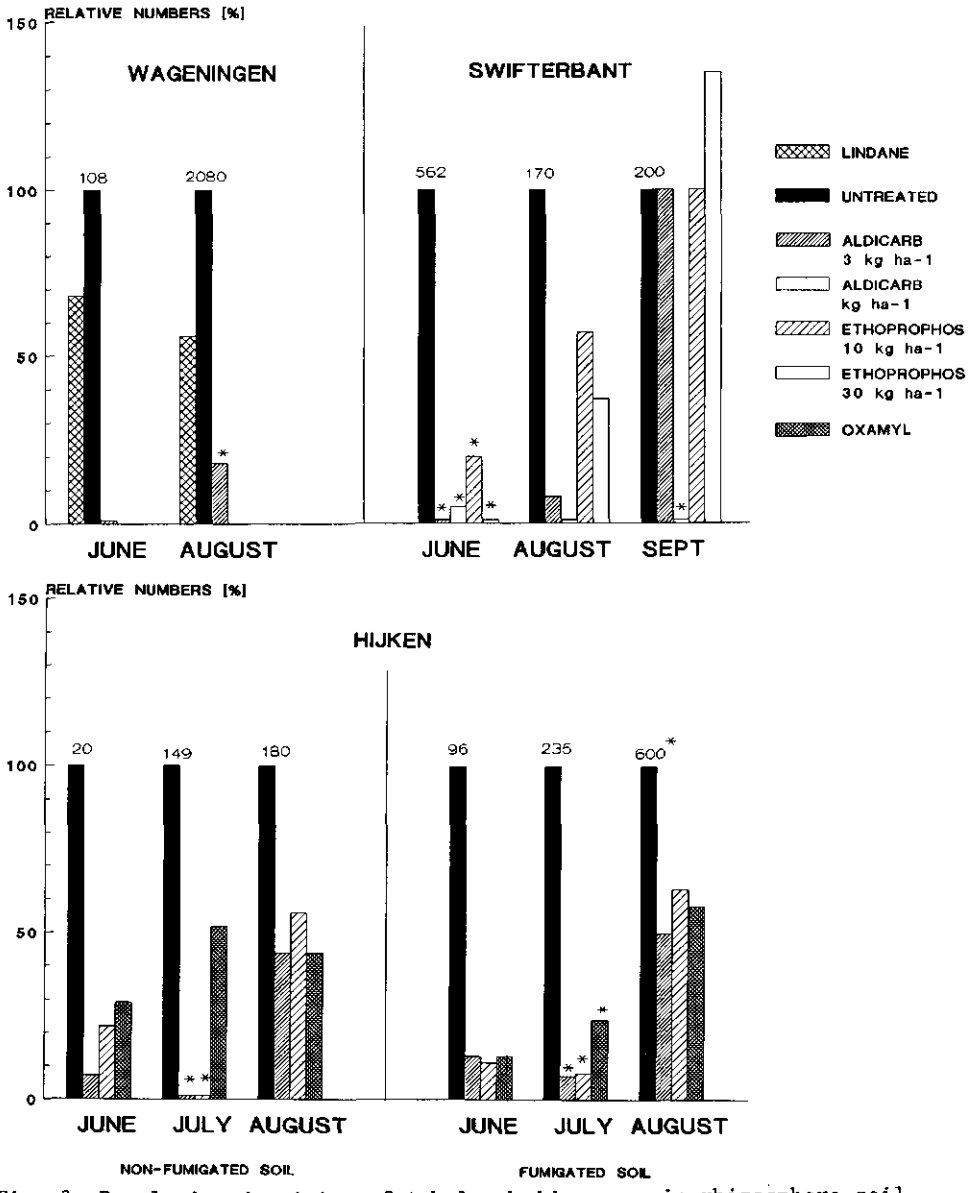

Fig. 3. Population densities of Aphelenchoides spp. in rhizosphere soil from plots treated with granular nematicides, lindane or the fumigant dichloropropene. Explanation and numbers as in Fig. 2.

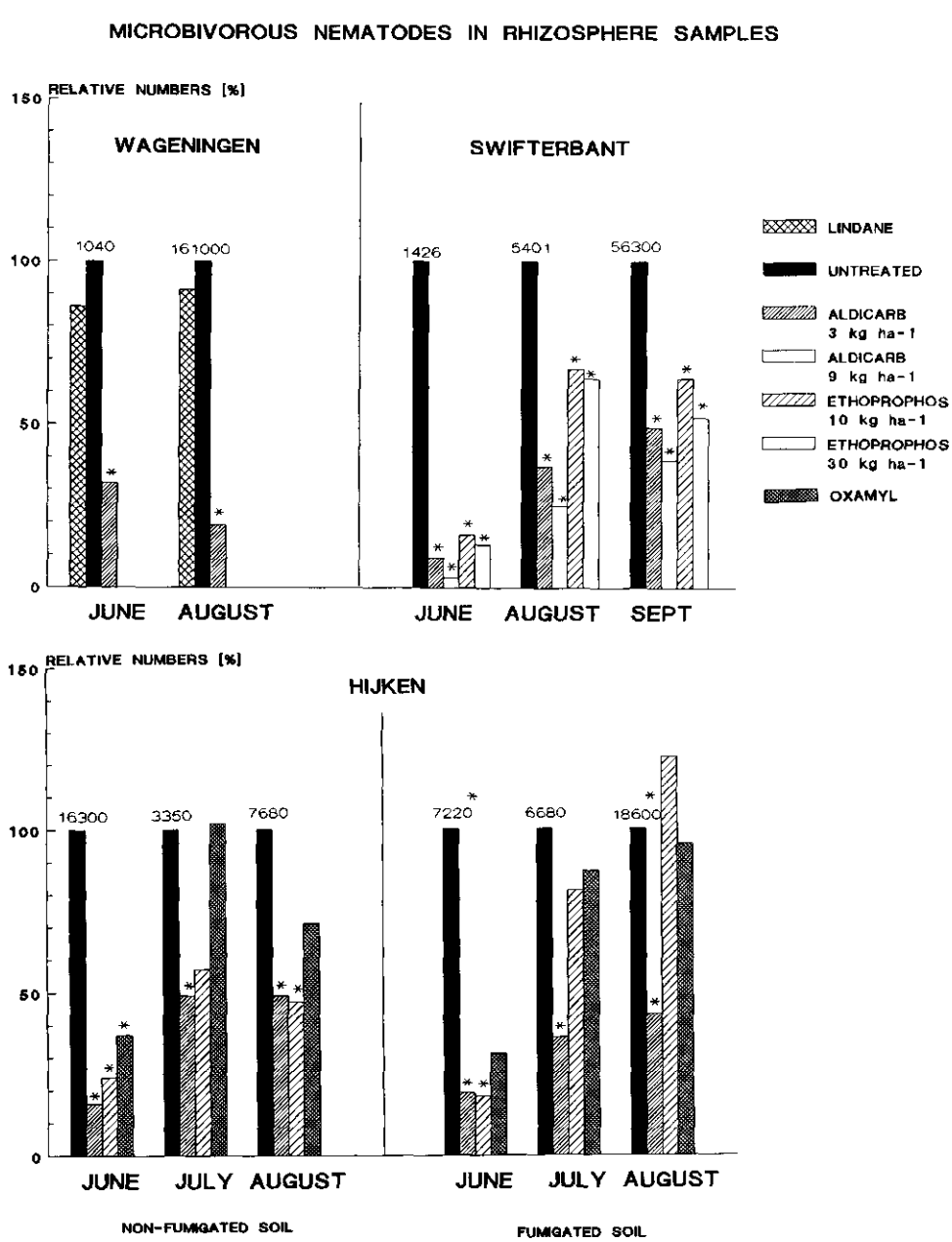

Fig. 4. Population densities of microbivorous nematodes in rhizosphere soil from plots treated with granular nematicides, lindane or the fumigant dichloropropene. Legend and numbers as in Fig. 2.

At the first sampling date, nematode numbers were equally reduced by ethoprophos, aldicarb and oxamyl in rhizosphere soil, but nematicides were less effective in the soil sampled by auger (Fig. 1 - 4). In all three fields A. avenae and Aphelenchoides spp. were more sensitive to nematicides than the microbivorous nematodes. At the second sampling date, the effects of nematicides were less than at the first sampling and the nematicidal effect of oxamyl was only small. In the fumigated field, ethoprophos was less active than in the non-fumigated field, while in both fields aldicarb caused a similar reduction of nematode populations. At the last two sampling dates, nematode numbers were higher in ethoprophos treated plots than in untreated plots of the fumigated field.

In the trial on clay at Swifterbant, the nematode populations were reduced most at the highest dosages of ethoprophos and aldicarb. As in Hijken, the nematicidal activity of ethoprophos decreased towards the end of the season.

In the field at Wageningen, lindane had no adverse effect on mycophagous and other nematodes and aldicarb showed comparable effects as to other fields.

Numbers of A. avenae in the different treatments in the rhizosphere of dying roots are plotted against black scurf incidence in these treatments (Fig. 5). The curve was calculated with the data from Hijken and Swifterbant on the assumption of an exponential relationship. The equation for the line is:

$y = 75.6\ x^{-0.2188} + 0.90$ y = black scurf incidence (%), x = number of A. avenae per g of dying roots at the final sampling. With low numbers of A. avenae a change in population density was related to a large difference in black scurf incidence. At nematode numbers above approx. 75 per g rhizosphere sample, the same change in nematode numbers was related to a smaller difference of black scurf incidence.

For the field at Wageningen, this relationship did not fit the data (Fig. 5). The average number of A. avenae in the untreated plots was 735 per g rhizosphere sample (Fig. 2) and the percentage of tubers with at least moderate occupation of black scurf was 8.2 % (Table 4). In aldicarb-treated plots, numbers of nematodes were 44 per g rhizosphere sample and black scurf incidence was 55 %. The incidence of black scurf

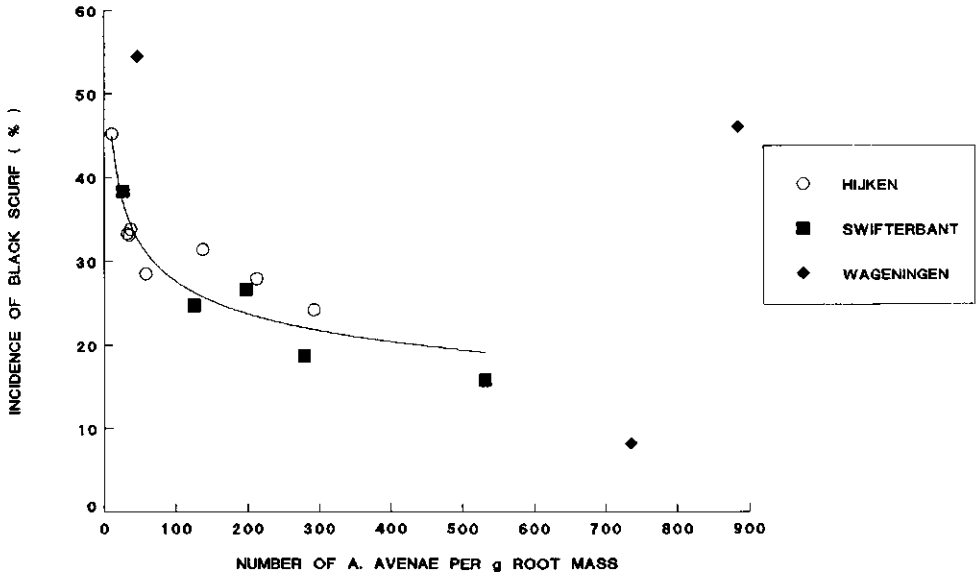

Fig. 5. The mean number of Aphelenchus avenae per g of dying root mass plotted versus the incidence of black scurf on tubers from soils treated with aldicarb, ethoprophos, oxamyl or lindane. The curve was calculated using data from the trials at Hijken and Swifterbant.

was more than expected on the basis of the same nematode density in the other fields. When plots had been treated with lindane, the numbers of A. avenae were 20 % higher than in samples from untreated plots, but the black scurf incidence was 46 %. The relationship between A. avenae and black scurf was therefore completely lacking in lindane-treated soil.

Flax straw baits. The numbers of nematodes extracted from the straws were highest at the second sampling date (Figs. 6 and 7). At the last sampling date, the numbers of nematodes had strongly decreased in both fields. This might have been due to the excessive rainfall during the second half of July. Microbivorous nematodes were much more abundant than A. avenae. Aphelenchoides spp. were less abundant than A. avenae (Chapter 4).

Ethoprophos and lindane did not significantly affect the nematode populations in the flax straws, while aldicarb almost completely eliminated A. avenae and strongly suppressed microbivorous nematodes.

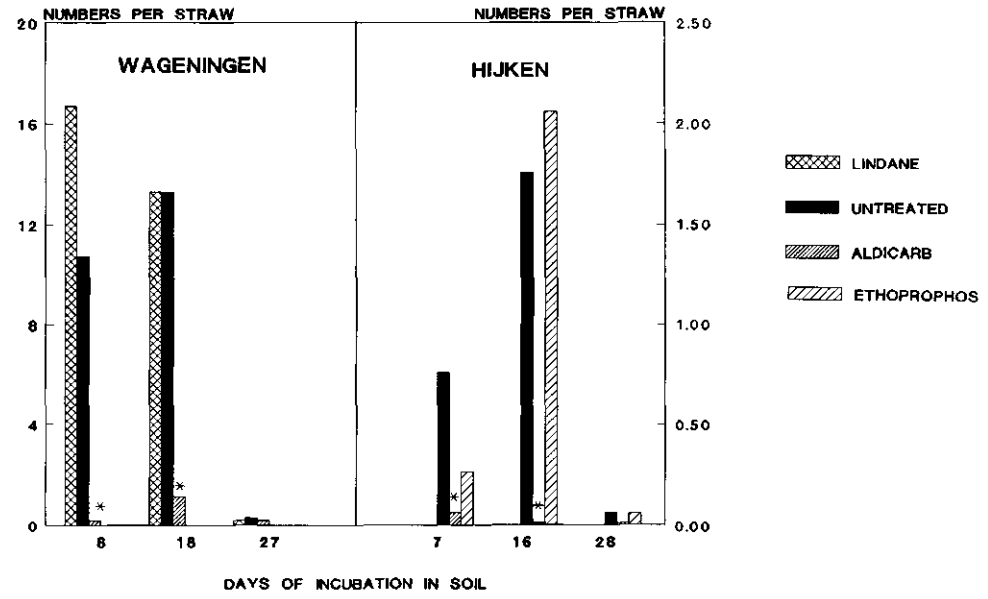

Fig. 6. Number of Aphelenchus avenae extracted from flax straws that were colonized by Rhizoctonia solani and buried in soil treated with aldicarb, ethoprophos or lindane at Hijken or Wageningen. Asterisks indicate a significant difference between numbers on straws in untreated and treated soil.

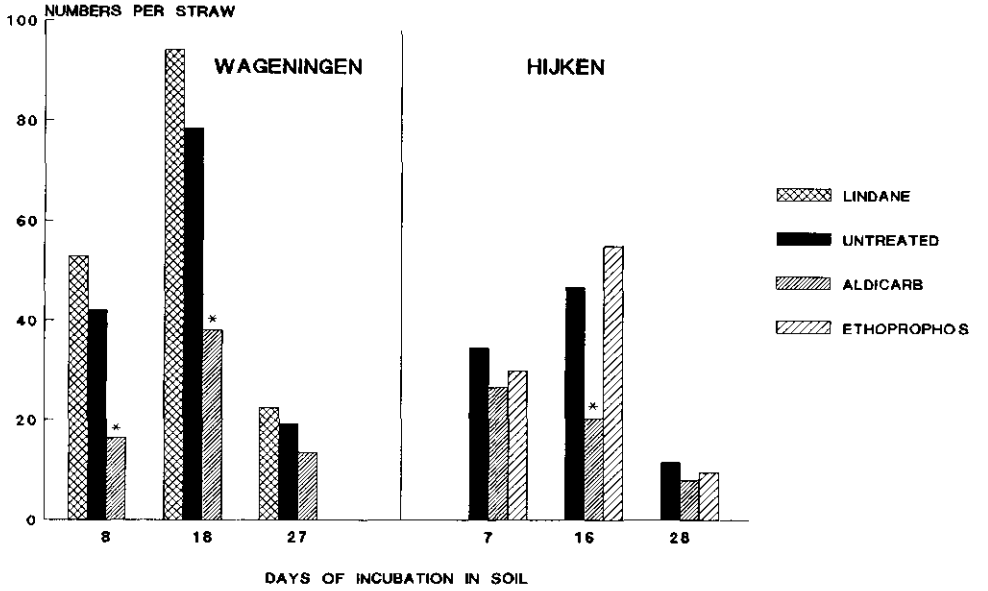

Fig. 7. Number of microbivorous nematodes extracted from flax straws that were colonized by Rhizoctonia solani and buried in soil treated with aldicarb, ethoprophos or lindane at Hijken or Wageningen. Otherwise as in Fig. 6.

Discussion

Effects of the pesticides on disease severity.

At the first sampling date in Hijken, the disease severity of potato stems was slightly higher in plots treated with dichloropropene, than in untreated plots (Table 2). However, at the later sampling dates, neither stem infection, nor black scurf were different between fumigated and non-fumigated plots. Dichloropropene can alter the nitrogen form in the soil resulting in a reduction in nitrate content with a concomitant increase of ammonia due to an inhibition of nitrifying bacteria (Elliot et al., 1974). Sanford (1947) showed that infection of potatoes by R. solani could be reduced both by adding nitrate or ammonium to the soil, nitrate being slightly more suppressive than ammonium. Therefore the increased disease severity at the first sampling in fumigated soil is, not explained by effects from an altered nitrate/ammonium ratio on disease.

At the beginning of the season it is possible that dichloropropene or metabolites formed after application still exerted some fungicidal action, because up to the planting date a characteristic smell was detected after disturbing the soil. This may have been favoured by the preceding long and cold winter.

At all locations, the stem infection by R. solani and incidence of black scurf was higher in plots treated with granular nematicides than in untreated plots. Aldicarb caused the strongest increase of stem infection and black scurf. The difference in disease severity between untreated and aldicarb-treated soil, was greatest in the field at Wageningen.

The increase in disease severity, i.e. larger and more frequent lesions, between the first and the second harvest confirms earlier observations, that potato stems can also become severely infected by R. solani after emergence (Hofman and Jongebloed, 1988). This was contrary to trials of Van Emden (1965), who reported a post-emergence resistance.

At Hijken, the granular nematicides promoted stem infection and black scurf more in non-fumigated than in fumigated soil. In a comparable experiment at Hijken in 1986 with the same soil and

cropping history, effects were similar to the effects reported here, i.e. the effects of granular nematicides on R. solani were much stronger in a non-fumigated field than in a field that had been fumigated with metham-sodium in the previous autumn (Hofman and s'Jacob, 1988).

In the trial on clay, effects of ethoprophos on R. solani were not significant. Laboratory trials (Chapter 3) have already shown that ethoprophos, when applied at higher dosages, reduced stem infection by R. solani, probably due to its fungitoxicity (Chapter 2). This is also indicated by the data on stem infection at Swifterbant.

On clay, the detoxification of ethoprophos and aldicarb was probably more rapid than in Hijken, because of a higher soil pH. The high pH may have caused an increased hydrolysis of the compounds (Van Berkum and Hoestra, 1979). This would also explain the absence of effects from aldicarb applied at the dosage of 3 kg a.i. per ha, which is recommended for field application.

Effects of pesticides on nematodes.

At the first sampling date at Hijken, all nematode numbers were higher in the non-fumigated than in the fumigated subfield (Figs. 1 - 4). Later in the season, numbers in the fumigated subfield were at least twice as high as in the non-fumigated subfield. It seems likely that the numbers increased more rapidly in the fumigated field, because predatory mites and springtails and those competing for food re-established themselves slower than the mycophagous nematode species. A slow re-establishment of important springtails and mites was observed and will be reported in a later paper (Chapter 6).

Nematode numbers (Fig. 1 - 4) show that aldicarb had the longest residual activity. Over the season, the nematicidal effect of ethoprophos and oxamyl decreased in all fields more rapidly. This may explain why ethoprophos only slightly increased black scurf and oxamyl had no effect. In the fumigated field at Hijken, A. avenae was more abundant in samples from ethoprophos-treated plots than in the untreated plots, probably due to a relatively smaller predatory fauna in these plots.

At the final sampling of the rhizosphere in the fumigated field at Hijken, the population density of A. avenae in the aldicarb-treated plots was 17 % of that found in the rhizosphere of untreated plots (Fig. 2). However, numbers of A. avenae were much higher in the fumigated field than in the non-fumigated field. This means that the remaining population was still 3700 ind. per 100 g root sample in aldicarb treated plots in the fumigated field. This density was approximately equal to that found in the rhizosphere of ethoprophos- and oxamyl-treated plots in the non-fumigated field. The incidence of black scurf in these three treatments was about the same, i.e. 33 % tubers with sclerotia in classes two or three (Table 2). The very high numbers of A. avenae can therefore explain the absence of effects in the fumigated field.

In Hijken and Swifterbant there seems to be a relationship between the numbers of A. avenae and incidence of black scurf (Fig. 5). The data from Wageningen demonstrate that the level of infection by R. solani cannot be explained by the activities of A. avenae alone. It is possible that A. avenae plays a more important role in fields at Hijken and Swifterbant than at Wageningen. In all fields, a significant contribution in the reduction of R. solani is probably due to the mycophagous activity of the mesofauna (i.e. mites and springtails). Mites and springtails were equally suppressed by lindane and aldicarb (Chapter 6).

Attempts have been made to use the mycophagous nematodes A. avenae and Aphelenchoides spp. for biological control of soil-borne pathogens (Barker,1964, Klink & Barker, 1968, Jouan et al., 1972, Roy, 1973 and Rössner & Urland, 1983). In most of these studies extremely high nematode numbers were required for an effective suppression of diseases on different host plants. This paper is, as far as is known, the first to report about relations between mycophagous nematodes and the incidence and disease severity of soil-borne pathogens in arable fields.

The results of the trial with flax straw baits also demonstrated that in ethoprophos treated plots, nematicidal activity was low from the second half of July, i.e. 3 months after application of the compound

(Figs. 6 and 7). Straws lifted from plots treated with lindane were colonized with slightly higher numbers of nematodes than those from untreated plots. This might be explained by reduced competition and predation by the mesofauna in lindane treated plots.

Aldicarb had a strong inhibitory effect on the population of A. avenae. This indicates, that when mycelium of R. solani is abundant, aldicarb can prevent A. avenae from grazing and reproducing on the mycelium. Therefore, due to the plant-pathogen interaction in an aldicarb-treated soil R. solani may colonize the host without any interference from A. avenae.

The inhibitory effect of aldicarb was less severe on microbivorous nematodes, than on A. avenae. No attempt was made to identify species of the microbivorous nematodes. It is, therefore, possible that most of the microbivorous species were eliminated, and that the population density of those species that are less sensitive to aldicarb had increased, because of reduced competition for nutrients.

Chapter 7 will deal with the quantitative relationship between some mycophagous organisms and stem infection of potatoes by R. solani. The effects of nematicides and lindane on mites and springtails will also be reported (Chapter 6). The results in this paper and previous ones indicate that the effects of nematicides on R. solani may be explained by a reduction of mycophagous nematodes, springtails and mites in nematicide- treated soil. Which species are most important in the suppression of R. solani, will depend upon the most abundant mycophagous species and the soil type.

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CHAPTER 6

EFFECTS OF ALDICARB, ETHOPROPHOS, LINDANE AND DICHLOROPROPENE ON MITES AND SPRINGTAILS IN POTATO FIELDS INFESTED WITH RHIZOCTONIA SOLANI

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Abstract

Previous inestigations indicated that mycophagous soil animals may be the most important factor explaining an increased infection of potatoes by Rhizoctonia solani in nematicide-treated fields. The effects of aldicarb, ethoprophos, lindane and dichloropropene were studied on springtails and mites in a number of potato fields on sand and clay. The fields were sampled twice, with a time interval of at least two months.

The most abundant species in the fields were the springtails Tullbergia krausbaueri, Isotoma notabilis and Folsomia fimetaria and the mites Pygmephorus sellnicki, P. blumentritti, Coccotydeus sp. and Histiosoma litorale.

The Pygmephorus spp. were slightly and T. krausbaueri moderately sensitive to aldicarb. Early in the growing season, numbers of T. krausbaueri were lower in aldicarb-treated plots than in untreated plots in two out of three experimental fields. In samples taken at the end of the growing season, numbers of T. krausbaueri were much higher in aldicarb-treated plots than in untreated plots in a field on clay. In two sandy fields, T. krausbaueri was still reduced or numbers were equal to untreated. Numbers of most other species of mites and springtails were low in samples from aldicarb-treated plots at both dates.

The effect of ethoprophos on the mesofauna corresponded with that of aldicarb. In the field on clay, mites and springtails were more numerous in ethoprophos-treated soil than in untreated soil at the second sampling date.

In order to distinguish between effects of mycophagous nematodes and the mycophagous mesofauna on R. solani lindane was applied to one field. It had no nematicidal activity, but as with aldicarb and ethoprophos, it increased disease severity of R. solani on potatoes. In lindane-treated plots, all species of mites and springtails, except predatory mites were reduced.

Dichloropropene almost eliminated all fauna species. However, by the end of July, most species present in non-fumigated plots had re-established themselves in the fumigated plots, with predatory mites being present in extremely high numbers.

The data support the hypothesis that mycophagous springtails and mites contribute to a lower disease severity of R. solani on potato stems and formation of sclerotia on tubers, because of grazing on mycelium of the pathogen.

Additional keywords: granular nematicides, Acari, Collembola, Tullbergia krausbaueri, Isotoma notabilis, Folsomia fimetaria, Pygmephorus spp., Coccotydeus sp., Histiosoma litorale, predatory mites, food preference.

Introduction

The pathogenic fungus Rhizoctonia solani Kühn has a wide range of host crops. Symptoms on potato are lesions on underground stems and stolons, and sclerotia (black scurf) on tubers. Spring application of the granular nematicides aldicarb, oxamyl and ethoprophos, increases disease severity and black scurf (Leach and Frank, 1982; Scholte, 1987; Hofman and s'Jacob, 1988; Chapter 5). The effect is probably due to an effect of the nematicides on mycophagous soil animals that can reduce the disease severity of R. solani (Hofman and Bollen, 1987; Hofman and Jongebloed, 1988; Chapter 5). The granular nematicides not only affect nematodes, but are also toxic to most other animals in the soil. Effects of the granular nematicides and of the insecticide lindane on mycophagous nematodes and on disease severity were reported in a previous paper (Chapter 5). This paper reports effects of aldicarb, ethoprophos, dichloropropene and lindane on the population of springtails and mites in relation to the disease severity of R. solani.

Some authors have reported that the mesofauna can reduce soil-borne diseases. In laboratory trials, Ulber (1983, 1986) introduced the springtail Folsomia fimetaria into soil resulting in almost complete control of Pythium root rot and infection of sugar beet by R. solani. Curl (1979) found that a population of equal numbers of the springtails Proisotoma minuta and Onychiurus encarpatus caused a strong reduction in the percentage of cotton plants infected by R. solani.

Materials and methods

Field characteristics. Samples were taken in three potato fields at different locations. An extensive description of field characteristics and planting is presented in a previous paper (Chapter 5).

1. Wageningen. This field was a sandy soil (pH-KCl 6.1 and organic matter content 3.3 %). It was divided into 18 plots, as a randomized complete block design with three treatments and six replicates. The treatments were: untreated, aldicarb (3 kg a.i. per ha, as Temik 10G) and lindane (1.47 kg a.i. per ha, as Chimac-Lindaan). Planting and application of the pesticides were carried out on 14 April 1987.

2. Swifterbant. This field was a calcareous marine clay (pH-KCl 7.2 and organic matter content 4.5 %). The composition of the soil was 25 % clay, 55 % silt and 20 % sand. The field was divided into 25 plots as a randomized complete block design with five treatments and five replicates. The treatments were: untreated, ethoprophos applied at 10 kg a.i. per ha (as Mocap 20 GS), ethoprophos applied at 30 kg a.i. per ha, aldicarb at 3 kg a.i. per ha (as Temik 10G on corncob grid) and aldicarb applied at 9 kg a.i. per ha. Planting and application of nematicides were carried out on 23 April 1987.
3. Hijken. This field was a sandy soil (pH-KCl 5.9 and organic matter content 6 %). Two subfields were situated next to each other. One of these two was fumigated on 3 November 1986 with dichloropropene (240 l ha^{-1} Telone II). Each of the two subfields was divided into 12 plots as a randomized block design with three treatments and three replicates. Treatments were: untreated, aldicarb (3 kg a.i. per ha, as Temik 10G) and ethoprophos (10 kg a.i. per ha, as Mocap 20 GS). Planting and application of nematicides were carried out on 21 April 1987.

Sampling of the mesofauna. All fields were sampled twice, using an auger (5.5 cm diam.). At the first sampling date, soil pellets were taken from a depth of 7.5 - 10 cm in each plot. Care was taken not to disturb the soil structure. The pellets were immediately placed on small sieves, which were subsequently transferred to an extraction apparatus.

There was a high rainfall in the summer of 1987, so the soil was very wet at the second sampling date. Therefore, at this date the soil was sampled at 2.5 - 5 cm deep instead of 7.5 - 10 cm, since we expected the mesofauna to have migrated upwards from the water saturated zone. At both depths, stems, stolons and some roots could be found, although the density of roots and stolons was highest at 7.5 - 10 cm. The samples were taken in the top of the ridge and as close as possible to the plants. When the sample contained a tuber, it was discarded.

In Wageningen, five subsamples per plot were taken on 9 June and 19 August 1987. In Swifterbant, four subsamples per plot were taken on 15 June and 16 September 1987. In Hijken, four subsamples per plot were

taken on 1 June and 31 July 1987. All samples were individually wrapped in small plastic bags and transported to the laboratory in an insulated box.

Extraction of the mesofauna. Small metal sieves containing the soil samples were placed on metal containers (10 cm high), in a tray which was cooled down to 15 °C with running tap water. The soil samples from the sandy fields were carefully poured onto a new sieve before placing them in the extraction apparatus. Since the sand was compressed during transport, this handling formed pores to enable the animals to move out.

Extraction in the Petersen extraction apparatus lasted six days (Petersen, 1978). The power supply to the light bulbs was manually adjusted with daily intervals so, that the temperature on the soil surface of the samples increased linearly from 20 to 40 °C.

Statistics. The number of mites and springtails in a treatment was assessed by combining the 4 or 5 subsamples per plot, each of approx. 60 ml. Therefore, the plot is only represented by a small amount of soil taken from a few selected sites. The subsamples often contained very different species and population numbers. For many species the variation between samples due to an aggregated distribution pattern was too large to detect significant differences between treatments. Other species had a more homogeneous distribution pattern. Therefore, the only suitable statistical analysis that could be used was a test on rank scores. For each species the rank scores over treatments were determined within blocks and analyzed with ANOVA. Differences between numbers in treated and untreated plots were assessed with Dunnett's-test.

Results

Experimental field at Wageningen. On both sampling dates, the most abundant springtails were Tullbergia krausbaueri (Börner), Isotomodes productus (Axelson), Folsomia fimetaria (L.) and at the second sampling date Hypogastrura denticulata (Bagnall) (Table 1). Collembola that were less frequently observed (less than 10 individuals per kg soil) were Proisotoma minuta (Tullberg), Sminthurinus aureus (Lubbock), Pseudosinella alba (Packard), Willemia anophtalma Börner, Onychiurus armatus (Tullberg), Friesea mirabilis (Tullberg), Brachystomella pavula (Schäffer), Entomobrya sp., Anurida pygmaea (Börner), Isotoma viridis (Bourlet) and Isotomina sp.. When a species was abundant in one subsample, but rarely found in other subsamples or plots. it was not mentioned in Table 1.

At the first sampling date, the number of most springtail and mite species was lower in lindane- and aldicarb-treated plots than in untreated plots. Numbers of T. krausbaueri, the most numerous species, were only slightly reduced by these agents at the first sampling date, but had equal numbers in all treatments at the second sampling date. In untreated soil, the same numbers of T. krausbaueri were detected at both sampling dates.

Hypogastrura denticulata had an aggregated distribution pattern. This species was only found in a few samples, but, if present, was very abundant. Differences between treatments were not statistically significant due to high variance in samples.

The most abundant mites were Pygmephorus sellnicki Krczal and Coccotydeus sp. Thor (Table 1). Depending on the sampling date Pygmephorus blumentritti Krczal, Pygmephorus silvestris Hirst, Alicorhagia sp. Berlese, Oppia minus (Paoli) and Tyrophagus sp. Oudemans were also observed in most of the samples, but they had an aggregated distribution pattern. Species that were present at low densities were Variatipes quadrangularis (Paoli), Pygmodispus calcaratus Paoli, Zygoribatula propinquus Oudemans, Oppia nova (Oudemans), Histiosoma litorale Kramer, Rhizoglyphus robini (Claparède), Tectocepheus velatus (Michael) and unidentified Eupodidae Koch, Rhagidiidae Oudemans and Nanorchestidae Grandjean.

Table 1 Springtails and mites in plots treated with lindane or aldicarb and untreated plots of a potato field at Wageningen (numbers per kg dry soil).

Springtails

Treatment	Tullbergia krausbaueri	Folsomia fimetaria	Isotomides productus	Hypogastrura denticulata	Other species	Total
9 June 1987						
Untreated	96	13	23	0	6	138
Lindane	36	2	5 *	0	1	44
Aldicarb	26	0 *	2 *	0	2	30
19 August 1987						
Untreated	103	5	25	8	15	156
Lindane	76	6	3 *	23	10	118
Aldicarb	132	8	4 *	17	73	234

Mites

Treatment	Pygmephorus spp.	Tyrophagus sp.	Coccotydeus sp.	Oppia spp.	Alicorhagia sp.	Other species	Total	Predatory species
9 June 1987								
Untreated	90	15	54	0	24	24	207	21
Lindane	14 *	0 *	18	0	3 *	15	50	17 *
Aldicarb	43	0 *	3 *	0	0 *	19	65	2 *
19 August 1987								
Untreated	10	0	21	21	4	37	93	28
Lindane	5 *	1	7	5	0	22	40 *	20
Aldicarb	16	0	8	15	1	14	54 *	9 *

Asterisks indicate significant differences to untreated plots ($p < 0.05$).

Pygmephorus spp. were moderately suppressed by aldicarb and more strongly by lindane. At the first sampling date, Coccotydeus sp. was suppressed more in aldicarb-treated plots than in lindane-treated plots. At the second sampling date, the numbers of Pygmephorus spp., Coccotydeus sp. and Tyrophagus sp. were much lower than initially.

The numbers of predatory mites, mainly Gamasides Leach, were equal in samples from both dates. Lindane hardly affected their number, while aldicarb caused a significant reduction.

Experimental field at Swifterbant. T. krausbaueri and Isotoma notabilis Schäffer were the predominant species among the Collembola (Table 2). Folsomia candida Willem and Neelus minimus (Willem) were present in lower numbers. Other springtails that were present in low

Table 2 Springtails and mites in plots treated with ethoprophos or aldicarb and in untreated plots of a potato field at Swifterbant (numbers per kg dry soil).

Springtails

Treatment	Tullbergia krausbaueri	Isotoma notabilis	Folsomia candida	Neelus minimus	Other species	Total
9 June 1987						
Untreated	130	237	32	4	24	427
Ethoprophos 1[1]	31 *	87 *	12 *	0 *	32	162 *
Ethoprophos 2[2]	39 *	31 *	4 *	1	1	76 *
Aldicarb 1[1]	123	22 *	0 *	0 *	1	146 *
Aldicarb 2[2]	88	1 *	0 *	0 *	2	91 *
16 September 1987						
Untreated	121	14	26	14	2	177
Ethoprophos 1[1]	138	12	70	13	4	237
Ethoprophos 2[2]	263 *	21	26	40 *	17	367 *
Aldicarb 1[1]	306 *	6	8	16	7	343 *
Aldicarb 2[2]	485 *	10	22	17	22	556 *

Mites

Treatment	Pygmephorus spp.	H.litorale[3]	Coccotydeus sp.	Oppia spp.	T.velatus	Eupodidae	Other species	Total	Predatory species
9 June 1987									
Untreated	7	1	25	34	55	3	59	184	32
Ethoprophos 1[1]	32 *	0	8	16 *	4 *	4	7	71 *	7 *
Ethoprophos 2[2]	8	1	5	7 *	13 *	0 *	1	35 *	4 *
Aldicarb 1[1]	48 *	0	1 *	2 *	5 *	0 *	4	61 *	0 *
Aldicarb 2[2]	156 *	0	0 *	0 *	0 *	0 *	5	161	1 *
16 September									
Untreated	1	17	42	18	1	28	33	140	42
Ethoprophos 1[1]	11	71	81	8	0	53	68	292 *	27
Ethoprophos 2[2]	15 *	19	143 *	31	0	52 *	55	315 *	48
Aldicarb 1[1]	10 *	45	13 *	4	0	21	35	128	21 *
Aldicarb 2[2]	26 *	35	12 *	2	0	5 *	22	102	19 *

[1]Application at recommended rate, i.e. ethoprophos at 10 kg a.i. per ha and aldicarb at 3 kg a.i. per ha.

[2]Application at three times the recommended rate.

[3]H. litorale = Histiosoma litorale, T. velatus = Tectocepheus velatus.

Asterisks indicate significant differences to untreated plots ($p < 0.05$).

numbers, often with an aggregated distribution pattern were Proisotoma minuta, Willemia anophtalma, Arrhopalites caecus (Tullberg), Onychiurus sp. Börner, Lepidocyrtus cyaneus Tullberg, Anurida pygmaea, Hypogastrura denticulata and Isotomina sphagneticola. The total number of springtails was much higher than in the field at Wageningen.

At the first sampling date, the total number of springtails was reduced in aldicarb- and ethoprophos-treated plots. The most abundant springtail species, Isotoma notabilis, was suppressed more in aldicarb-treated plots than in ethoprophos-treated plots. Numbers of T. krausbaueri were not affected by aldicarb applied at the recommended dosage. Numbers of T. krausbaueri were suppressed in ethoprophos-treated plots, and also to a small extent in plots treated with aldicarb at a high dosage. Folsomia candida was absent in samples from aldicarb-treated plots and significantly suppressed in ethoprophos-treated plots.

The second sampling showed completely different effects of the nematicides on the mesofauna. In untreated plots the numbers of T. krausbaueri were comparable with the initial numbers. In nematicide-treated plots, especially in those treated with aldicarb, a significantly higher number of T. krausbaueri was observed. The numbers were highest in plots that had received the highest dosages of nematicides. Of I. notabilis only low numbers were observed and effects of nematicides were absent. Due to a high variation between numbers in different plots, numbers of F. candida and Neelus minimus were not significantly different between treatments.

The most abundant mites were Pygmephorus sellnicki, Tectocepheus velatus, Coccotydeus sp., Oppia minus, Histiosoma litorale and an unidentified species belonging to the Eupodidae.
Other mites, present in low numbers, were Pygmephorus blumentritti, Oppia nova, Zygoribatula propinquus, Alicorhagia sp., Rhizoglyphus sp. and some Rhagidiidae. The population densities of the Pygmephorus spp. were much higher in plots treated with nematicides than in untreated plots. Aldicarb applied at three times the recommended dosage resulted in the strongest increase in numbers. The other mite species were only rarely observed at the first sampling in aldicarb-treated plots and had significantly lower numbers in ethoprophos-treated plots than in untreated plots. Predatory mites were also eliminated by aldicarb and

Table 3 Springtails and mites in plots treated with dichloropropene, and/or ethoprophos or aldicarb and untreated plots of a potato field at Hijken. (numbers per kg dry soil).

Springtails

Treatment		Tullbergia krausbaueri	Willemia anophtalma	Folsomia fimetaria	Other species	Total
1 June 1987						
No	Untreated	114	21	7	5	147
dichloro-	Ethoprophos	0 *	0 *	0 *	0	0 *
propene	Aldicarb	20 *	1 *	0 *	0	21 *
dichloro-	Untreated	1	1	0	0	2
propene	Ethoprophos	0	0	0	0	0
	Aldicarb	0	0	0	1	1
31 July 1987						
No	Untreated	301	52	174	6	533
dichloro-	Ethoprophos	3 *	0 *	2 *	0	5 *
propene	Aldicarb	89 *	27 *	13 *	0	129 *
dichloro-	Untreated	29	89	1	7	126
propene	Ethoprophos	0 *	0 *	1	1	2 *
	Aldicarb	0 *	0 *	0	2	2 *

Mites

Treatment		Pygmephorus spp.	Histiosoma litorale	Coccotydeus sp.	Oppia spp.	Other species	Total	Predatory species
1 June 1987								
No	Untreated	1	0	7	27	9	44	15
dichloro-	Ethoprophos	4	0	0 *	0 *	0	4	0 *
propene	Aldicarb	26	0	0 *	1 *	2	29	0 *
Dichloro-	Untreated	4	0	0	0	0	4	1
propene	Ethoprophos	3	0	0	0	0	3	0
	Aldicarb	1	0	0	0	2	3	0
31 JULY 1987								
No	Untreated	89	88	31	25	25	258	6
dichloro-	Ethoprophos	47	42	31	0 *	5	125 *	9
propene	Aldicarb	34	9	3	0 *	5	51 *	4
Dichloro-	Untreated	121	8	31	61	13	234	91
propene	Ethoprophos	89	5	2 *	0 *	8	104	67
	Aldicarb	120	2	0 *	0 *	6	128	11 *

Asterisks indicate significant differences to untreated plots ($p < 0.05$).

strongly reduced by ethoprophos.

At the second sampling date, the numbers of Coccotydeus sp., Eupodidae and Pygmephorus spp. were higher in ethoprophos-treated plots than in untreated plots. The numbers of Pygmephorus spp. were higher in aldicarb-treated plots than in untreated plots. However, Coccotydeus sp., Oppia spp., Eupodidae and predatory mites (mostly Gamasides) were less numerous in aldicarb-treated plots than in untreated ones.

Experimental field at Hijken. The most abundant species were the springtails T. krausbaueri, Willemia anophtalma, F. fimetaria, and the mites Pygmephorus spp., H.litorale, Coccotydeus sp. and Oppia nova (Table 3). Other species that were recorded, but always at numbers lower than 10 individuals per kg soil, were the springtails Willemia aspinata Stach, Arrhopalitus caecus, Lepidocyrtus cyaneus, Sminthurinus aureus, Proisotoma minuta, Entomobrya sp. and the mites Tectocepheus velatus, Tyrophagus sp., Zygoribatula cognatus, Rhizoglyphus robini, Alicorhagia sp., and species belonging to the Eupodidae, Rhagidiidae and Tydeidae Kramer. The Pygmephorus spp. consisted of approximately equal numbers of P. blumentritti and P. sellnicki.

At the first sampling date, very few springtails and mites were observed in plots that had been fumigated with dichloropropene. The number of mites did not differ appreciably between fumigated and non-fumigated plots at the second sampling date, but most of the springtails were still less numerous in the fumigated plots. The number of predatory mites was extremely high in fumigated plots. Some of the mites and springtails that had re-established themselves in the fumigated soil were found mainly in the two blocks that were bordered by a non-cultivated weedy path and that also had a higher moisture content than the other two blocks, viz. 16 % and 12 %, respectively. The species most observed in these plots were T. krausbaueri, Willemia anophtalma, Coccotydeus sp. and Oppia nova. These species were not recorded in the two blocks furthest from the path.

In ethoprophos- and aldicarb-treated plots, numbers of all springtails and mites were reduced. Ethoprophos was more effective than aldicarb against springtails, while aldicarb was more effective against

mites. At the first sampling date, mite counts were too low and the distribution pattern of species was too aggregated to enable definite conclusions to be drawn. Only O. nova and predatory mites had homogeneous distribution patterns. Histiosoma litorale had a highly aggregated distribution pattern, so differences were not significant, although the results suggest that aldicarb reduced the population. In the fumigated field, W. anophtalma was the most abundant springtail species and Pygmephorus spp. and O. nova were the most prevalent mite species. Pygmephorus spp. were the only species that re-established themselves in plots treated with granular nematicides.

Discussion

Granular nematicides significantly reduced the numbers of mycophagous nematodes in the rhizosphere of potato plants as was reported in Chapter 5. The results reported here demonstrate that this applies also to mites and springtails, but that the species differ in their sensitivity.

At the second sampling dates, due to the high moisture content of the soil, samples were taken less deep. The difference in depths at the two dates, may partly explain the difference in mesofauna populations. It was assumed that the wetness of the soil would result in an upward migration of the mesofauna, but it seems likely that not all species react similar to an increase in moisture content of the soil. If the soil moisture content did not have a large influence on the distribution, some of the species that were abundant in the second samplings (from 2.5 - 5 cm deep), could have been abundant at this depth at the first sampling dates as well, but then a deeper soil layer was sampled. Since the location of mites and springtails in soil is dependent on moisture, soil structure, gaseous compounds, temperature (fluctuations), subterranean plant parts and other organic material, it is not possible to study the population dynamics with the information obtained at the two sampling dates. However, the samples provide information on the effects of the pesticides on the mesofauna at the time of sampling.

The reproduction rate is an important factor for the rate at which a species can recolonize the soil. When the concentration of the nematicide drops below a level of no-effect, species with a low reproduction rate will remain at a low density for a longer period than those with a high reproduction rate. T. krausbaueri and Pygmephorus spp. were more numerous in most of the nematicide-treated plots than in untreated plots in the Swifterbant field. This may have been due to a combination of a high reproduction rate with a reduced population of predators, and a reduced competition for food with other members of the mesofauna. Mycophagous and other microbivorous nematodes, which have high reproduction rates, were still strongly reduced in aldicarb-treated plots at the last sampling dates (Chapter 5). This indicates that aldicarb was still active, and that T. krausbaueri and Pygmephorus spp. are probably less sensitive to aldicarb.

Predatory mites were not affected by lindane, so in lindane-treated plots numbers of other springtails and mites may have been limited by predation to the same extent as in untreated plots.

In a previous paper it was shown that lindane increased infection of R. solani to almost the same extent as aldicarb (Chapter 5). In plots treated with lindane, the population of mycophagous nematodes was equal in numbers to those in untreated soil, so it was concluded that the main suppression of R. solani was probably due to the activities of the mesofauna in the Wageningen field. The samples from this field contained the lowest total mesofauna population of all the fields tested, while the difference in disease severity between treated plots and untreated plots was larger than in the other fields. During the season it became clear that the seed tubers had been planted deeper under the top of the ridge in the Wageningen field than in the other fields, so less roots were present in the samples. As with mycophagous nematodes (Chapter 5) springtails and mites are most abundant in the rhizosphere (Wiggins et al., 1979). Sampling was carried out at one depth in all fields, so it is possible that numbers were more abundant at deeper soil levels in the field at Wageningen. Species of springtails and mites also have different feeding patterns, so the composition of the population is important for the extent to which R. solani is reduced.

T. krausbaueri was the most abundant species in all fields. It was possible to rear T. krausbaueri on cultures of R. solani, so this springtail may reduce severity of the disease caused by this fungus in the field (Chapter 7). During the growing season R. solani colonizes subterranean stems, stolons and tubers. At the first sampling date, lower numbers of T. krausbaueri were observed in nematicide-treated plots, so in these plots R. solani could grow better and could have formed more sclerotia at the end of the season. In the field at Hijken, T. krausbaueri was strongly reduced at both sampling dates in nematicide-treated plots in the non-fumigated field, while disease severity was significantly increased in these plots. In the Wageningen field numbers of T. krausbaueri were equal in all treatments at the second sampling date and in the Swifterbant field, numbers of T. krausbaueri were much higher in nematicide-treated plots at the second sampling date. It is possible that the higher numbers later in the season compensated the initially reduced grazing on R. solani in nematicide-treated plots.

Pygmephorus spp. were also quite abundant in most plots. Kosir (1975) found that Pygmephorus mesembrinae and P. quadraticus could be reared on some fungi, but not on those belonging to the Basidiomycetes to which Class R. solani belongs. He also found that the two species had a preference for different fungi. It is possible that the Pygmephorus species found here are not feeding on R. solani, but mainly on other organisms. Than disease severity will not be affected by these mites. Aldicarb and ethoprophos did not reduce numbers of Pygmephorus spp. so it would appear that these mites are not important in the reduction of R. solani. Moreover, it was impossible to rear them on plate or pure soil cultures of R. solani (Chapter 7).

A few microarthropods were successfully isolated from the field and reared on cultures of R. solani (Chapter 7). Of these F. fimetaria was the only species that was able to reduce the disease severity of R. solani at low population densities. With five other species very high numbers were needed to reduce disease severity. However, not all species that were abundant in the three fields were included in these laboratory trials, so it is possible that other species are also

efficient in reducing infection by R. solani .

No selective sampling of microarthropods in the rhizosphere was made, as was done with nematodes. It is well possible that some of the species that were observed at low numbers were almost exclusively living in the rhizosphere. They might have been more important than species that were abundant in the samples and not specifically grazing on the plant surface but e.g. on dead organic material. Since aldicarb, ethoprophos and lindane reduced most of the springtails and mites and increased disease severity by R. solani, this supports the hypothesis that some of these soil animals reduce the inoculum. Species that were not reduced in number or even increased in plots where aldicarb, ethoprophos and lindane had increased disease severity, were Hypogastrura denticulata, Histiosoma litorale (only in the field at Swifterbant) and Pygmephorus spp.. Species observed in the samples that may have played a role in the suppression of R. solani were T. krausbaueri, F. fimetaria, Isotomodes productus, Coccotydeus sp., Alicorhagia sp., Isotoma notabilis, Folsomia candida, Oppia spp., Tectocepheus velatus, Willemia anopthalma and Histiosoma litorale, because their numbers were reduced at one or both sampling dates in nematicide-treated soil.

In the experimental field at Hijken, soil fumigation with dichloropropene almost completely eliminated the mesofauna. The fumigant dichloropropene does not have a residual toxicity during the next growing season. Therefore, species with a high reproduction rate can rapidly increase to numbers higher than observed in untreated soil because of a lack of competitors and predators. Willemia anophtalma, Pygmephorus spp., Oppia nova and mycophagous and other microbivorous nematodes were more abundant in samples from fumigated soil at the second sampling date than in samples from non-fumigated soil. Heungens and Van Daele (1974) observed that fumigated greenhouse soil was recolonized by the mesofauna within one year after treatment. Edwards and Lofty (1969) reported that it took more than 2 years after fumigation with dichloropropene before populations were the same between treated and untreated fields. In fumigated plots in the Hijken field, F. fimetaria was the only species not recorded at the end of July, when compared with the species that were numerous in the non-

fumigated plots.

In the second sampling, the total population of springtails and mites was lower in the fumigated plots than in the non-fumigated plots, but there was an unexpectedly large population of predatory mites in the fumigated soil. The large number of predators indicate an abundance of prey. The total number of mites was equal in both fumigated and non-fumigated plots, but the total number of springtails was much lower in the fumigated plots. However, numbers of nematodes were more than twice as high in fumigated plots than in non-fumigated plots (Chapter 5). The predatory mites, therefore, probably live mainly on nematodes. Another explanation of the large predator population in fumigated soil could be that they not only feed on mites, springtails and nematodes, but also on the soil microflora or dead organic material. In this case they may gain from the limited number of competitors for food in the soil.

In fumigated plots at Hijken at the first sampling date, the absence of the mycophagous fauna suggested that the disease severity would be higher in the fumigated plots than in non-fumigated plots. However, the disease severity was the same in these plots at all sampling dates (Chapter 5). It is possible that dichloropropene altered the microbial antagonism to R. solani over a longer time, although this was not shown in antibiosis tests (as in Hofman and Bollen, 1987). The long, cold preceding winter might have led to dichloropropene or some metabolites formed after application still being present in the soil at planting. When the soil was disturbed a characteristic smell was released from the fumigated soil. During the first days after planting, this may have caused some reduction of the inoculum of R. solani. It was not surprising that by absence of the mycophagous fauna in the fumigated field effects of aldicarb and ethoprophos on disease severity were not detected.

The results of the samplings of the mesofauna demonstrate that as the numbers of most springtails and mites decrease in soil treated with aldicarb, ethoprophos or lindane, the infection with R. solani increases. All mycophagous animals in soil not treated with nematicides may jointly contribute to a reduction of the infection of potato stems by R. solani and formation of black scurf. Of these F.

fimetaria seems to be one of the most important mycophagous species.

Laboratory trials on the quantitative relationship between a number of mycophagous nematodes, springtails and mites, and disease severity of R. solani on potato stems are presented in Chapter 7.

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CHAPTER 7

QUANTITATIVE RELATIONSHIPS BETWEEN DISEASE SEVERITY OF RHIZOCTONIA SOLANI ON POTATOES AND SOME MYCOPHAGOUS SOIL ANIMALS

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Abstract

In an attempt to explain the increased disease severity in nematicide-treated fields, the effects of nematodes, springtails and mites on infection of potatoes by Rhizoctonia solani were studied in laboratory experiments. The species studied were the nematodes Aphelenchus avenae and Aphelenchoides sp., the springtails Folsomia fimetaria, Tullbergia krausbaueri and Arrhopalites caecus and the mites Rhizoglyphus robini, Histiosoma litorale and Oppia nova. All species were isolated from potato fields.

The mycophagous animals were reared in soil on cultures of Alternaria porri. Together with inoculum of R. solani on wheat grains, they were gently mixed through coarse sand. Potato tubers of cv. Bintje were planted in this soil. After incubation for three to four weeks at 15 °C, stems were assessed for disease symptoms.

The disease severity was strongly reduced by F. fimetaria, and also, although slightly less, by A. avenae, T. krausbaueri, R. robini and A. caecus. Aphelenchoides sp., O. nova and H. litorale had little effect on disease.

Among the species tested F. fimetaria, T. krausbaueri and A. avenae were the most numerous ones in Dutch potato fields. A few mites, Pygmephorus spp., were also numerous, but they did not reproduce on mycelium of R. solani or Alternaria porri in vitro.

In the laboratory trials, the densities of mycophagous animals needed to reduce disease severity were higher than those observed in most fields. However, in each experiment only one species was tested, whereas in field soil, the effects of different mycophagous animals may be additive. The experiments demonstrate that under favourable conditions, some of the species can reduce disease severity in the field.

Additional keywords: Aphelenchus avenae, Aphelenchoides sp., Folsomia fimetaria, Tullbergia krausbaueri, Arrhopalites caecus, Rhizoglyphus robini, Oppia nova, Histiosoma litorale, stem infection, pencycuron, mycophagous fauna, nematodes, mites, Acari, springtails, Collembola.

Introduction

The soil fauna can reduce incidence of soil-borne diseases (Rhoades and Linford, 1959; Barker, 1964; Klink and Barker, 1968; Jouan et al., 1972; Roy, 1973; Curl, 1979; Ulber, 1983, 1984 and 1986). An increased disease severity of Rhizoctonia solani Kühn on potatoes grown in fields treated with the insecticides/nematicides aldicarb, ethoprophos and oxamyl, and with the insecticide lindane was reported in Chapter 5. These results confirmed observations of other authors on increased disease severity of R. solani on potato after application of aldicarb (Leach and Frank, 1982) and ethoprophos or oxamyl (Scholte, 1987). It has been reported in previous papers that a reduced activity of the mycophagous mesofauna may play an important role in the increased disease severity in nematicide treated soil (Hofman and Bollen, 1987; Hofman and Jongebloed, 1988; Hofman and s'Jacob, 1988; Chapters 5 and 6)

In a study on the various stages of the infection process by R. solani on potato, it was observed that the size of lesions was proportional to the size of the infection cushions (i.e. masses of swollen hyphae) on the plant surface (Hofman and Jongebloed, 1988). Therefore any factor influencing the growth of R. solani and the size of infection cushions will affect the size of the lesions and so influence the severity of the disease.

Mycophagous animals feed by grazing on the mycelium or by sucking out the contents of hyphae. They may therefore reduce disease severity by decreasing the amount of vital mycelium or by disturbing transport through the hyphae. Conversely, some diseases may increase because the mesofauna spreads fungal inoculum (Shew and Beute, 1979; Manzer et al., 1984). R. solani was found to be a suitable nutrient source for the reproduction of the springtails Onychiurus fimatus (Ulber, 1980) and O. encarpatus and Proisotoma minuta (Wiggins and Curl, 1979). The animals did not spread the fungus through the soil. Other springtails and mites may also not disperse R. solani, because the fungus does not form spores in the soil and its mycelium consists of broad hyphae, that are difficult to consume as whole cells by small soil animals.

When R. solani has already formed infection cushions, the fauna can reduce the size before penetration of the fungus in the host occurs. The effect of a partially destroyed infection cushion on the size of lesions is unknown. Large infection cushions may cause only small or no lesions when some of the hyphae are broken, because nutrient flows are disturbed.

Nematodes and mesofauna (i.e. mites and springtails) may differ in their ability to reduce mycelium or infection cushions. The most common mycophagous nematodes Aphelenchus avenae Bastian and Aphelenchoides spp. Fischer are able to reproduce parthenogenetically very rapidly on R. solani (Mankau and Mankau, 1963; Barker 1964; Nickle and McIntosh, 1968; Rössner and Nagel, 1984). This means that nematodes can increase rapidly on an infection cushion which may result in a reduction of the lesion size. However, it is possible that infection has occurred before the nematodes are numerous enough to reduce the inoculum. Lesions can appear within five days after inoculation at 16 °C (Hofman and Jongebloed, 1988).

Species of the mesofauna are larger and more mobile than nematodes. When an individual mite or springtail arrives at an infection cushion it probably eats tiny pieces at random. Mites and springtails are not able to multiply so rapidly as nematodes when food sources become available. Mites and springtails generally already migrate before all the food is consumed at a certain location (personal observation).

The aim of this study was to determine quantitative relationships between the most common mycophagous soil fauna species in certain Dutch potato fields and the severity of disease caused by R. solani. Only species that could be reared on the fungi R. solani or Alternaria porri Ellis were included in the trials.

Materials and methods

Sampling methods. Mites and springtails were extracted from soil with an electronically operated apparatus according to Andrén (1985). Extractions lasted 18 h, during which the temperature at the top of the 60 ml soil samples increased from 20 °C to 40 °C. Mites and springtails were collected in 25 % picric acid. When mites and springtails had to be collected alive for propagation purposes, the collecting boxes were

filled with water.

Nematodes were extracted from the soil with an Oostenbrink elutriator (Oostenbrink, 1960). Nematode suspensions were poured over nematode filters, which were placed overnight in a collecting tray in 100 ml of tap water to wet the filter.

Nematode numbers in the cultures on A. porri were assessed by placing 10 g of a well mixed culture on a nematode filter in a collecting tray with 100 ml tap water for 24 h (three replicates).

At the end of one of the trials, the stem surface of some diseased stems was also examined for the presence of A. avenae. Numbers of nematodes on diseased and healthy parts of stems were assessed by placing stem parts of 1 cm length on nematode filters in water.

All nematode numbers were assessed in two 5 ml subsamples from the collecting tray suspensions and counted under a dissecting microscope.

Rearing of mycophagous organisms. After isolation of nematodes, mites or springtails from potato fields, the species were transferred to potato dextrose agar covered with mycelium of R. solani or Alternaria porri. Species that increased their numbers on the fungal colonies were reared in soil on a pure culture of A. porri. This fungus was used, because it is not pathogenic to potato and does not affect the incidence and severity of disease. Potting soil (organic matter content 70 %) was mixed with 5 % oat meal (w/w). This mixture was brought up to a moisture level of 73 % of fresh weight and autoclaved twice on two subsequent days (120 °C, 20 min). The sterilized substrate (100 ml in a 300 ml flask) was inoculated with Alternaria porri.

When the soil had been colonized by the fungus for 14 days, nematodes, mites or springtails were introduced. The cultures were incubated in the dark at 20 °C. After several generations had been produced, the cultures were used for inoculation experiments.

Organisms brought into culture with this procedure were the nematodes A. avenae, Aphelenchoides spp.; the springtails, Folsomia fimetaria Linné, Tullbergia krausbaueri Börner and Arrhopalites caecus Tullberg, and the mites Rhizoglyphus robini Claparède, Histiosoma litorale Oudemans and Oppia nova Oudemans. The time needed to reach high numbers in the cultures differed considerably between the species. Nematodes increased to very high numbers within 7 days.

Cultures with mites or springtails had to be started 1 to 5 months before they could be used in the inoculation experiments. The change in population densities during the inoculation experiments was expressed by the ratio between inoculated and final population density (P_f/P_i).

Rhizoctonia inoculum. R. solani was grown on sterilized wheat grains. The grains were autoclaved twice in water on two subsequent days. The water was poured off and the moist grains were then inoculated with R. solani and incubated at 20 °C. The isolate of R. solani AG-3 originated from a sclerotium on a potato tuber from sandy soil.

When cultures were approx. three weeks old, the grains were dried in the airstream of a sterile cabinet for one day and then made into a meal by dry milling. In all experiments, an inoculum dosage of 0.5 g of the meal per litre soil was used.

Seed tubers. Seed tubers of cv. Bintje, 25 - 28 mm diam., were used. Before planting, tubers, maximum of one year old, were transferred from cold storage (4 °C) to room temperature and placed in the light for 7 - 12 days, to activate sprouting. Tuber-borne sclerotia of R. solani were killed before planting by dipping the seed tubers in a 0.09 % solution of the antibiotic validamycin (Solacol).

Inoculation experiments. Tubers were placed at the bottom of plastic boxes 4 x 4 cm and 20 cm high. Each treatment consisted of 20 replicates.

Coarse sand was used in all experiments, since there are almost no micro-organisms in this substrate. The inoculum of R. solani and the mycophagous organisms were carefully mixed with the soil in a plastic bag. The mycophagous animals were added with the Alternaria-cultures to minimize mechanical damage. In the control treatments and in treatments with low numbers of animals, additional potting soil was added to the sand in order to obtain the same soil composition in all treatments. All trials with mites and springtails were started with two different population densities, the higher initial density being three times as high as the lower density.

The soil was brought up to a moisture content of 10 % (w/w). Boxes

were placed in the dark at 15 °C. When stems had emerged from most of the boxes (i.e. after about 3 weeks), they were examined for disease severity.

In one experiment a treatment with the fungicide pencycuron (Moncereen L 25 %) was included to compare the decrease in disease caused by A. avenae with a fungicide having high activity against mycelial growth of R. solani. The dosage of pencycuron was 0.0018 ul l^{-1}, which is only 0.5 % of the dosage recommended for use in sandy field soil.

Disease severity rating. The experimental unit was one box. Within a box the stems were rated according to the size of the lesion: 0, stems without lesions; 1, small lesion present; 2, medium sized lesion; 3, stem girdled by a lesion; 4, stem cut off, because of severe infection. The disease severity per tuber was calculated with an index:

$$\text{Index} = \frac{\sum_{i=0}^{i=4} i \cdot x_i}{4 \cdot \sum_{i=0}^{i=4} x_i} \cdot 100\ [\%]$$

i = disease assessment class

x_i = number of stems per class

In the results the average disease index is reported as well as the incidence of all stems with lesions in a treatment.

Statistics. Statistical analysis was carried out with Wilcoxon's one-sided two sample test, which was conducted on the index values per tuber (= one box). Comparisons were made between untreated controls and the treatments with the highest numbers of mycophagous animals (one sided testing). When the difference was significant, the lower population density was also tested with the same test. A trial with A. avenae was an exception to this procedure, because all treatments were tested against the control.

Results

The fungus Alternaria porri, on which the mycophagous soil animals were reared, had no influence on the infection of the stems by R. solani.

Severe infection of the primary stems resulted in development of many secondary stems. Therefore, the number of stems was much higher in the treatments without animals, where disease severity was higher than in most of the treatments with nematodes, springtails or mites. As a consequence, the number of stems could influence the disease rating. It was expected that the disease severity on secondary formed stems would be less than on primary stems, because of a later start in development. However, it was observed that when secondary stems were formed after the main stem was killed, the newly formed stems were also severely infected by R. solani.

Table 1. Effects of Aphelenchus avenae on incidence of Rhizoctonia solani on potato stems, disease index and number of stems per tuber.

Initial population (numbers/l)	Population at harvest (numbers/l)	P_f/P_i	Disease incidence(%)	Disease index(%)	P-value[4]	Number of stems/tuber
Experiment 1[1]						
Control	-	-	96	84	-	2.4
10,000	40,000	4.0	28	10	0.000	2.4
100,000	400,000	4.0	48	18	0.0003	2.1
pencycuron[3]	-	-	2	1	.000	2.3
Experiment 2[2]						
Control	-	-	75	62	-	3.0
500	3,800	7.6	69	42	0.035	2.6
1000	9,900	9.9	83	52	0.20	2.4
3000	33,100	11.0	68	40	0.056	2.5
5000	56,400	11.3	75	51	0.15	2.5

[1] Experiment 1 was started on 15 April and incubated for 22 days.
[2] Experiment 2 was started on 28 August and incubated for 19 days.
[3] Pencycuron was applied at a rate of 0.0018 ul l^{-1} soil.
[4] The p - values for significance were obtained by comparing the disease indexes between inoculated treatments and untreated with Wilcoxon's two sample test (one-sided analysis).

The nematode A. avenae reduced disease incidence and index of stems infected (Table 1). In the second experiment, where low numbers of nematodes were added, the incidence of disease was not reduced by A. avenae, but significant effects were found on disease severity. This was mainly due to the fact that fewer stems were killed by the fungus.

The lowest number of A. avenae, that could be mixed and resulted in good dispersion throughout the soil was 500 individuals (ind.) per litre sand. Even this rate resulted in a reduction of disease severity.

In the first experiment six stems with lesions of classes 2 and 3 were examined for the distribution of A. avenae. The stems were divided into two subsamples of three. A diseased part and a healthy part both of 1 cm length were taken from each stem. The numbers of nematodes extracted from the two subsamples were 380 and 70 from the diseased parts, and 30 and 10 from the healthy parts of the same stems. The difference supports the hypothesis that A. avenae can live on the mycelium of R. solani on the surface of stems and that it rapidly increases in population density when food becomes available. In the first experiment numbers of A. avenae had increased four-fold during the incubation period, which shows that R. solani is a very suitable nutrient source for reproduction of this nematode (Table 1).

The fungicide pencycuron applied at a very low dosage was much more effective in decreasing stem infection by R. solani than A. avenae at all the population densities investigated.

Table 2. Effects of Aphelenchoides sp. on incidence of Rhizoctonia solani on potato stems, disease index and number of stems per tuber.

Initial population (numbers/l)	Population at harvest (numbers/l)	P_f/P_i	Disease incidence(%)	Disease index(%)	P-value[3]	Number of stems/tuber
Experiment 1[1]						
Control	-	-	34	18	-	1.6
10^4	400	0.04	47	22	-	1.6
10^5	2050	0.02	35	24	-	1.2
10^6	-	-	12	3	0.084	1.7
Experiment 2[2]						
Control	-	-	92	90	-	3.6
5,000	1800	0.36	86	82	-	3.5
10,000	1400	0.14	85	80	-	3.4
20,000	1600	0.08	92	87	-	3.2

[1] Experiment 1 was started on 4 February and incubated for 26 days. Inoculation was carried out with R. solani on perlite instead of milled wheat kernels.
[2] Experiment 2 was started on 23 June and incubated for 27 days.
[3] p - values as in Table 1.

Aphelenchoides sp. was not as effective in decreasing the disease as A. avenae (Table 2). In the first experiment inoculation was carried out with R. solani on perlite and inoculum was prepared as in Hofman and Jongebloed (1988) instead of using an inoculum in milled wheat

Table 3. Effects of Folsomia fimetaria on incidence of Rhizoctonia solani on potato stems, disease index and number of stems per tuber.

Initial population (numbers/l)	Population at harvest (numbers/l)	P_f/P_i	Disease incidence(%)	Disease index(%)	P-value[3]	Number of stems/tuber
Experiment 1[1]						
Control	-	-	96	98	-	2.6
2100	1015	0.48	74	75	0.0001	2.5
3400	1060	0.31	26	31	0.0000	2.2
Experiment 2[2]						
Control	1000	-	87	89	-	4.2
429	1050	2.45	79	76	0.016	4.8
1800	3110	1.73	66	62	0.0009	5.0

[1] F. fimetaria was reared in soil/oatmeal culture on R. solani and not as the animals in other experiments on Alternaria porri. The experiment was started on 19 May and incubated for 22 days.
[2] Experiment was started with a 2 month old culture on 20 October and was incubated for 27 days.
[3] p-value as in Table 1.

Table 4. Effects of Tullbergia krausbaueri on incidence of Rhizoctonia solani on potato stems, disease index and number of stems per tuber[1].

Initial population (numbers/l)	Population at harvest (numbers/l)	P_f/P_i	Disease incidence(%)	Disease index(%)	P-value[2]	Number of stems/tuber
Control	-	-	76	70	-	3.4
5800	3130	0.54	70	68	0.43	3.4
9800	3660	0.37	53	51	0.047	2.1

[1] Experiment was started with a 2½ month old culture on 30 November and was incubated for 38 days.
[2] p - values as in Table 1.

Table 5. Effects of Arrhopalites caecus on the incidence of Rhizoctonia solani on potato stems, disease index and number of stems per tuber[1].

Initial population (numbers/l)	Population at harvest (numbers/l)	P_f/P_i	Disease incidence(%)	Disease index(%)	P-value[2]	Number of stems/tuber
Control	-	-	83	86	-	3.9
400	378	0.94	80	77	0.129	3.6
2260	360	0.16	86	65	0.0015	2.1

[1] Experiment was started with a 1 month old culture on 30 November and was incubated for 38 days.
[2] p - values as in Table 1.

grains. This explains the low disease incidence in the control treatment. In this trial, reduction of disease was only observed at a nematode density of one million nematodes per litre soil.

In the second experiment introduction of 5000 ind. per litre only slightly reduced the disease. However, using higher numbers did not result in an additional reduction. At the termination of the experiment, population densities were equal in the three different treatments and much lower than at the starting date of the experiment. This shows that R. solani was a poor nutrient source for Aphelenchoides spp.

In the treatment with Folsomia fimetaria, disease incidence and index were significantly reduced at 3400 ind. per litre soil (Table 3). The disease severity was intermediate at 2100 ind. per litre soil. In the second trial, lower initial numbers were used and disease suppression was less pronounced. At the termination of the trials in three out of four treatments the final population was approx. 1050 ind. per litre soil, but this density was also found in the boxes where no springtails were added! This indicated that F. fimetaria shows rapid dispersal.

Tullbergia krausbaueri decreased the disease index to almost the same extent as F. fimetaria, but the numbers needed for this disease reduction were much higher (Table 4). Disease suppression was apparent with 9800 ind. per litre soil and not with 5800 ind per litre. At the end of the experiment the numbers were the same in both treatments, i.e. 3400 per litre soil. These numbers were much lower than initially present.

Arrhopalites caecus significantly reduced the disease index at 2260 ind. per litre soil, but had no influence on disease incidence (Table 5). At the end of the experiment the numbers were reduced to 370 per litre soil in both treatments.

Table 6. Effects of a mixture of Rhizoglyphus robini (89 %) and Histiosoma litorale (11 %) on the incidence of Rhizoctonia solani on potato stems, disease index and number of stems per tuber[1].

Initial population (numbers/l)	Population at harvest (numbers/l)	P_f/P_i	Disease incidence(%)	Disease index(%)	P-value[2]	Number of stems/tuber
Control	-	-	100	98	-	2.2
600	720	1.20	95	94	0.016	2.1
1640	3040	1.85	100	70	0.0001	1.5

[1] Experiment was started with a 3 month old culture on 21 January and was incubated for 42 days.
[2] p - values as in Table 1.

A mixed population of Rhizoglyphus robini (89 %), and Histiosoma litorale (11 %) gave a good reduction in the disease index but did not affect disease incidence (Table 6). Both mites are not very abundant in potato fields (Chapter 6), but they are very common colonizers of fungi in laboratory collections. The final numbers showed that R. robini maintained its initial population density well, but only very few numbers of H. litorale were recovered.

Table 7. Effects of Oppia nova on the incidence of Rhizoctonia solani on potato stems, disease index and number of stems per tuber[1].

Initial population (numbers/l)	Population at harvest (numbers/l)	P_f/P_i	Disease incidence(%)	Disease index(%)	P-value[2]	Number of stems/tuber
Control	-	-	92	85	-	2.5
827	750	0.91	87	88	-	1.8
2240	2610	1.17	95	77	0.21	2.1

[1] Experiment was started with a 4 month old culture on 13 January and was incubated for 37 days.
[2] p - values as in Table 1.

Oppia nova did not affect disease incidence or index (Table 7). The population density at the end of the experiment was similar to the initial density in both treatments.

Discussion

The disease severity in treatments without animals, differed between the experiments. This was mainly due to the quality of the potato seed tubers. Seed tubers that were used after May, had no dormant buds and developed numerous thin stems. These stems might have been easier to sever by R. solani than larger stems from seed tubers of younger physiological age. The consequence of this was that the values of disease incidence and index were not comparable between experiments.

A. avenae reduced the infection of potato stems by R. solani. The lowest inoculum density of A. avenae used was 500 nematodes per litre soil, resulting in almost the same degree of disease suppression as larger numbers. Since an increase in the numbers did not result in an additional disease reduction, it is suggested that R. solani maintains a certain level of infection in presence of grazing nematodes. The numbers of A. avenae showing reduction of disease (500 ind. per litre soil) are much lower than the numbers needed for disease control of R. solani on bean as were found by Klink and Barker (1968).

In the second experiment, the multiplication rate of A. avenae became higher with increasing initial numbers. It can be concluded that even at 100,000 nematodes per litre soil, there was no limitation of food for A. avenae, because the multiplication rate did not decrease with increasing initial numbers.

A numerous initial population of A. avenae may reduce the colonization of the sterilized soil by R. solani or may reduce the size of infection cushions. In the second experiment the incidence of stem infection was not influenced by the presence of nematodes, but the disease index was. Here it is most likely that the nematodes reduced the size of infection cushions, but not the inoculum in the soil. In the first experiment, both disease incidence and index were reduced at high nematode numbers. This could be explained by both a reduction of inoculum density and a reduced development of infection cushions.

Under field conditions up to 735,000 ind. of A. avenae per kg decaying roots were detected (Chapter 5). The populations had increased rapidly when food became available. This means that from A. avenae a significant contribution in disease suppression can be expected in the field.

Aphelenchoides sp. poorly reduced disease severity. In the first experiment a reduction of disease severity was observed at 10^6 ind. per litre soil, but lower numbers were ineffective. In the second experiment, a small reduction of the disease index was obtained with 5000 ind. per litre. Higher numbers had no additional effect. In the field, the highest numbers of Aphelenchoides sp. found were 5600 per kg in the rhizosphere soil from potato plants on clay and 20800 per kg in the rhizosphere in sandy soil (Chapter 5). Although different species may be involved, these nematodes will probably contribute only very slightly to a reduction of disease severity in the field.

Other authors were more successful in their attempts to suppress fungal diseases using Aphelenchoides sp.. A. composticola at 100,000 ind. per litre soil strongly reduced disease of Fusarium oxysporum in melon (Cayrol, 1972). Rössner and Urland (1983) reduced infection of Fusarium culmorum and Gerlachia nivalis on wheat by introducing approx. 2,500 ind. of an Aphelenchoides sp. to the soil. It probably depends very much on the species whether disease suppression is achieved.

The strong decrease of the nematode population density during the experiment also shows that this species of Aphelenchoides cannot be expected to play an important role in the suppression of R. solani. Contrary to A. avenae, which had a high multiplication rate in all experiments, the population density of Aphelenchoides sp. decreased with increasing initial numbers. This indicates that for Aphelenchoides sp. adequate food is limited (i.e. mycelium availability).

It is possible that the final population density of Aphelenchoides sp. was not determined by the quantity of mycelium of R. solani in the soil, but by the mycelial mass of other fungi, present as contaminants.

Folsomia fimetaria strongly decreased the disease but only at densities that were higher than those found in field soil (Chapter 6). In inoculation trials, Ulber (1983) found that addition of approx. 120 ind. of F. fimetaria per kg soil resulted in an almost complete control of Pythium root rot of sugar beet. The behaviour of R. solani on potato is completely different from that of Pythium on beet and the trials also differ with respect to planting time. Ulber introduced sugar beet plants to the soil 5 days later than springtails and the inoculum. In my trials planting and inoculation were carried out on the same day. This might explain the higher disease suppressive activity of F. fimetaria to Pythium than to R. solani. Furthermore other factors such as soil type and type of infection process may be involved.

The highest numbers of F. fimetaria gave the greatest reduction of disease. This may be due to prevention by F. fimetaria of R. solani to colonize the plant or by feeding on infection cushions, since both the incidence of disease and index were reduced. Curl (1979) found with the springtail Proisotoma minuta, which is morphologically rather similar to F. fimetaria, and with Onychiurus encarpatus that 2000 ind. per kg soil of a population with equal numbers of both species, caused a smaller fraction of cotton plants being infected by R. solani, than when 1000 ind. per kg were introduced in field soil. In both treatments the effects were highly significant. When the field soil used was sterilized, higher numbers were needed for reduction of the disease. At 1600 ind. per kg there was no effect on disease, but 4000 ind. per kg resulted in complete control of R. solani. Lemaire et al. (1972) observed the opposite effect with A. avenae on R. solani and found less disease in sterilized soil than in field soil at equal population densities. It is possible that for control of R. solani on potato, lower numbers are needed in the field than in laboratory trials.

Ulber (1986) found that F. fimetaria gave a very strong suppression of infection of sugar beet by R. solani after the introduction of on average 7.5 springtails per plant. These numbers are difficult to compare with those in the pathosystem, with potatoes but as the numbers needed for disease control of R. solani for beet are rather low this appears promising for effects on R. solani on potato.

Springtails need larger pores in the soil through which to migrate than R. solani. This means that at lower population densities there will be a great quantity of mycelium of R. solani in the soil that can not be reached by the springtails, because it is growing within inaccessible soil pores. The final number of springtails in the soil is determined by the amount of R. solani and other fungi growing in the accessible soil pores. The maximum population density of F. fimetaria that could feed in these experiments is not clear, since an error in the assessment of the population density was caused by migration of the springtails. In one experiment, it was observed that non-inoculated pots contained approx. 1000 ind. of F. fimetaria per litre soil at 4 weeks after inoculation. The same number was found at harvest in the two inoculated treatments. At higher initial numbers of springtails, even when there is insufficient food available in the soil, disease severity will be further reduced, because the chances for R. solani to form undisturbed infection cushions will be reduced.

Tullbergia krausbaueri reduced disease at 9800 ind. per kg soil. This population density is however much higher than ever observed in the field (Chapter 6). When adding 5800 ind. per kg, which is also more than ten times as high as field populations, there was no effect on disease severity.

The mite Rhizoglyphus robini and springtail Arrhopalites caecus only occur in low numbers in the potato fields. R. robini is a very common contaminant in our mycological collections and does not show much food preference. Although both R. robini and A. caecus reduced disease severity in inoculation experiments, a substantial contribution of these species is not to be expected in the field.

The most important mites observed in potato fields are Pygmephorus spp. and Coccotydeus spp. (Chapter 6). So far we have been unable to rear them on R. solani and A. porri. Kosir (1975) found with two Pygmephorus species, that both were very selective in feeding behaviour. P. mesembrinae was less selective than P. quadratus in feeding on different fungi, but none of them fed on Basidiomycetes, the class to which R. solani belongs. Although we extracted other Pygmephorus species from the soil than Kosir, their food specialization may explain our difficulties in rearing them on fungi.

Oppia nova was found in our potato fields at low population densities. Results from the inoculation trial indicate that it probably does not play a role in reducing disease severity in the field, although it could maintain its initial population density during the length of the experiment, i.e. 37 days. This does of course not prove that this mite also feeds on R. solani.

In conclusion, it appears that F. fimetaria and A. avenae are the most important soil animals to cause a reduction in disease severity of R. solani in Dutch potato fields. To a lesser extent T. krausbaueri can also play a role. Mites probably play no role in the natural suppression of R. solani. Interactions between different fauna species will influence the disease reduction of various mycophagous species in an agricultural soil ecosystem.

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SUMMARY AND GENERAL DISCUSSION

The granular nematicides aldicarb, oxamyl and ethoprophos often are applied to control plant parasitic nematodes. However, the use of these pesticides may have some disadvantages. In field trials, they increased stem infection of potatoes caused by Rhizoctonia solani Kühn and incidence of black scurf (sclerotia of R. solani) on tubers. This thesis, reports about possible mechanisms involved in the increased infection in nematicide treated fields. The effects of granular nematicides were studied on:

- the pathogenicity of R. solani (Chapters 2 and 3)
- the susceptibility of the host plant (Chapter 3)
- the microbial antagonism to R. solani (Chapter 2)
- the mycophagous soil fauna, i.e. nematodes (Chapters 5 and 7) or springtails and mites (Chapters 6 and 7)

Ethoprophos was fungitoxic to R. solani. On Czapek Dox agar (CDA), the EC_{50} of the compound was 49 mg l^{-1} and at 100 mg l^{-1} there was a total growth inhibition. Oxamyl showed a slight fungitoxicity. At 100 mg l^{-1} oxamyl in CDA, the growth of R. solani was reduced by 26 %. Aldicarb did not influence the growth of R. solani on CDA, but caused a slight growth stimulation on potato dextrose agar (PDA).

The carbamate compounds aldicarb and oxamyl are systemically transported in plants. The presence inside the plant might, therefore, have some effect on the host-plant resistance to R. solani. The organophosphorus compound ethoprophos is not known to have a systemic activity, so it differs from the other two nematicides with respect to the uptake by the host plant. It was therefore, less likely that the increased infection by R. solani in nematicide-treated soil, was due to a reduced host-plant resistance. Laboratory experiments supplied the evidence that the nematicides did not reduce host-plant resistance.

Ethoprophos caused an initial growth inhibition of potato sprouts, but immediately after their emergence, growth was promoted. In laboratory experiments carried out at 15 °C in a growth chamber, the emergence of sprouts was delayed by three days, when tubers were planted in ethoprophos-treated soil. However, most probably due to the fungitoxicity of ethoprophos, the appearance of lesions on the stems was delayed by nine days in this treatment. Oxamyl showed similar

effects to ethoprophos, but to a lesser extent, while aldicarb did neither influence sprout development nor the appearance of lesions.

The stages in the infection process, including growth of runner hyphae, branching of hyphae on the stem surface, formation of infection cushions and the relationship between size of the infection cushions and size of lesions, were not influenced by any of the nematicides.

The previous observations made an indirect effect highly evident. In a study on the infection process was found that the size of lesions was proportional to the size of the infection cushions (dense masses of mycelium from which R. solani penetrates the plant). Any agent that changes the size of infection cushions will alter, therefore, the severity of the disease.

By far the most important mycoparasite of R. solani in Dutch potato fields is Verticillium biguttatum, so the study on effects on mycoparasites was focussed on this fungus. The growth of V. biguttatum was strongly stimulated by aldicarb and ethoprophos on potato dextrose agar plates covered with mycelium of R. solani. The mechanism involved was not studied. In the nematicide-treated fields, the incidence of V. biguttatum on stolons was increased. This may be due to an increased availability of substrate (i.e. mycelium of R. solani) or a reduced activity of the mycophagous soil fauna, as well as from a direct stimulation by the nematicide. At the recommended dosages of aldicarb, oxamyl or ethoprophos, an effect on soil fungistasis was not found, neither in laboratory experiments nor in field experiments.

The laboratory experiments indicated that the most probable explanation for the increased disease severity of R. solani in nematicide-treated fields is a suppression of the mycophagous soil fauna. In the experimental fields, the most abundant mycophagous nematodes were Aphelenchus avenae and Aphelenchoides spp. These nematodes could be reared on petri-dishes covered with mycelium of R. solani. In the rhizosphere of plants of which the foliage was removed, numbers of A. avenae increased dramatically. Outside the rhizosphere, only very low numbers of A. avenae and Aphelenchoides spp. were found. It was assumed that the large increase was due to a rapid development of the microflora in the rhizosphere of dying roots. Aldicarb, oxamyl and ethoprophos greatly limited this increase.

In a field that had been fumigated with dichloropropene, the granular nematicides hardly affected stem infection and black scurf incidence. Initially, metabolites that may have been formed after fumigation could have had a toxic effect on R. solani. All soil animals were strongly reduced at the beginning of the growing season in the fumigated field. Later in the season, numbers of A. avenae were much higher in the rhizosphere of plants grown in fumigated soil than in non-fumigated soil. Although the granular nematicides reduced numbers of A. avenae also in the fumigated soil, the remaining population density of A. avenae was still high. In nematicide-treated plots, the disease suppression was, therefore, not much lower than in the untreated plots.

To simulate potato stems colonized by R. solani, flax straws colonized by the fungus were buried in the field. Mycophagous nematodes rapidly increased on the straws. In aldicarb-treated plots, only low numbers of mycophagous nematodes were observed on these straws. These data show that grazing of mycophagous nematodes on mycelium and probably also on infection cushions of R. solani will be less in nematicide-treated soil than in untreated soil.

In one field trial, the effect of the insecticide lindane on R. solani was assessed to discriminate between the role of mycophagous nematodes and mycophagous microarthropods. In lindane-treated plots, stem infection and black scurf incidence were increased to almost the same extent as in aldicarb-treated plots. Lindane did not have any nematicidal activity. This indicates that at least in this field microarthropods may also reduce R. solani by grazing. Aldicarb, ethoprophos and lindane reduced the numbers of most springtails and mites in potato fields. The most abundant springtails were Tullbergia krausbaueri, Isotoma notabilis and Folsomia fimetaria, and the most abundant mites were Pygmephorus sellnicki, Pygmephorus blumentritti, Coccotydeus sp. and Histiosoma litorale. Numbers of Pygmephorus spp. were not reduced in aldicarb- and ethoprophos-treated plots. T. krausbaueri was reduced in lindane- and ethoprophos-treated plots, but only slightly in aldicarb-treated plots.

In the laboratory, T. krausbaueri, F. fimetaria and H. litorale could be reared on petri-dishes covered with R. solani or cultures of R. solani in sterilized soil. Pygmephorus spp. and Coccotydeus sp.

could not be reared on these cultures, while I. notabilis was not successfully isolated from the field. In laboratory experiments where the microarthropods were introduced in a soil inoculated with R. solani, T. krausbaueri and F. fimetaria caused a strong reduction of stem infection of potatoes. Lowest numbers of T. krausbaueri and F. fimetaria that significantly reduced disease severity were 9800 and 430 individuals per litre soil, respectively. H. litorale did not survive during the experiment, so it was probably not important in grazing on mycelium of R. solani. In similar inoculation experiments, A. avenae was also found to cause a significant reduction in stem infection when inoculated with 500 nematodes per litre. An Aphelenchoides sp. had no effect on stem infection. Nematodes reproduced within a few days at room temperature, while springtails and mites had a much slower reproduction cycle.

A. avenae and F. fimetaria seem to be some of the most important mycophagous soil animals that lead to a reduction by R. solani in the field. A smaller contribution in the reduction of disease severity can be expected from T. krausbaueri and other mycophagous soil animals that are only present at low densities. However, some of the species that were not reared in the laboratory on mycelium of R. solani, may also be able to cause a significant reduction of disease severity at low population densities.

Any pesticide that reduces the density of mycophagous soil animals will, therefore, facilitate a better development of R. solani. The stimulatory effects of granular nematicides on R. solani on potato seem primarily caused by reduced grazing of the mycophagous soil fauna on the pathogen in nematicide-treated fields.

SAMENVATTING

In de landbouw worden de granulaire nematiciden aldicarb, oxamyl en ethoprofos toegepast om schade door plantparasitaire nematoden te voorkomen. Toepassing van de middelen in de aardappelteelt kort voor het poten van aardappelen kan echter leiden tot een verhoogde mate van aantasting door Rhizoctonia solani Kühn en een zwaardere bezetting van knollen met sclerotiën (lakschurft). In dit proefschrift wordt het onderzoek beschreven naar mechanismen die betrokken zouden kunnen zijn bij de verhoogde mate van aantasting. De effecten van de nematiciden werden bestudeerd op:

-de pathogeniteit van R. solani (Hoofdstukken 2 en 3),

-de vatbaarheid van de waardplant (Hoofdstuk 3),

-het microbiële antagonisme tegen R. solani (Hoofdstuk 2),

-en de mycofage bodemfauna, te weten nematoden (Hoofdstukken 5 en 7) en springstaarten en mijten (Hoofdstukken 6 en 7).

Ethoprofos bleek fungitoxisch te zijn voor R. solani. De EC_{50} van ethoprofos op Czapek Dox Agar (CDA) was 49 mg l^{-1} en bij 100 mg l^{-1} was de groeiremming volledig. Oxamyl was in geringere mate ook fungitoxisch; bij 100 mg l^{-1} in CDA was de groei met 26 % gereduceerd. Aldicarb vertoonde geen effect op de groei van R. solani op CDA, maar een geringe groeistimulatie op aardappel-dextrose agar (PDA).

De carbamaten aldicarb en oxamyl zijn stoffen welke systemisch getransporteerd worden in de plant. De aanwezigheid in de plant zou dus een invloed kunnen hebben op de resistentie van de plant tegen R. solani. Ethoprofos, dat tot de organofosfaten behoort, heeft, voor zover bekend, geen systemische werking. Het werkings-mechanisme met betrekking tot de opname door de plant verschilt dus van de carbamaten. Hieruit is af te leiden dat het niet erg waarschijnlijk is dat een verhoogde aantasting door R. solani door nematiciden in het veld berust op een verhoogde gevoeligheid van de plant. In laboratoriumproeven werd aangetoond, dat de nematiciden de resistentie van aardappelstengels niet verminderden.

Ethoprofos veroorzaakte een initiële groeiremming van aardappelkiemen, maar zodra de spruiten boven het grondoppervlak uitgroeiden, was er sprake van een groeistimulatie. In inoculatie-experimenten, uitgevoerd bij 15 °C, bleek in ethoprofos-behandelde

grond de opkomst van aardappelkiemen met drie dagen vertraagd te zijn. De lesies op de stengels kwamen negen dagen later tot ontwikkeling dan in onbehandelde grond. Dit was waarschijnlijk het gevolg van de fungitoxiciteit van het middel. Oxamyl veroorzaakte dezelfde effecten als ethoprofos, maar in een geringere mate. Aldicarb had geen effect op de ontwikkeling van kiemen of op het verschijnen van lesies.

Het infectieproces zelf, met als stadia de groei van loophyfen, de karakteristieke vertakkingen van deze hyfen op het spruitoppervlak, de vorming van infectiekussentjes en de relatie tussen oppervlak van de infectiekussentjes en de grootte van de lesies, werd door geen van de nematiciden beïnvloed.

De bovengenoemde waarnemingen geven aan dat een indirect effect van de nematiciden op R. solani het meest waarschijnlijk was. In een studie van het infectieproces van R. solani op aardappelspruiten werd aangetoond dat de grootte van lesies afhankelijk was van het oppervlak van de spruit dat bedekt was met infectiekussentjes Een infectiekussentje is een dichte mycliummat van waaruit R. solani de plant binnendringt. Elke beïnvloeding van de grootte van het infectiekussentje betekent dus een verandering van lesiegrootte.

De effecten van de nematiciden op mycoparasieten werden alleen op Verticillium biguttatum bepaald. Dit is in Nederlandse akkers veruit de belangrijkste mycoparasiet van R. solani. Op PDA schalen werd de groei van V. biguttatum over mycelium van R. solani sterk gestimuleerd door aldicarb en ethoprofos. Er is niet nader ingegaan over de wijze waarop de groeistimulatie van de mycoparasiet tot stand komt, het verschijnsel kan niet uitsluitend worden toegeschreven aan een directe groeistimulatie door de nematiciden. In met nematiciden behandelde velden bleek dat Verticillium biguttatum op stolonen vaker werd aangetroffen dan in niet met nematiciden behandelde velden. Deze toename zou echter, naast een directe stimulering door de nematiciden, toegeschreven kunnen worden aan een verhoogde beschikbaarheid van substraat (d.w.z. mycelium van R. solani) voor de mycoparasiet of een verminderde begrazing van de mycoparasiet door de mycofage bodemfauna.

De bodemfungistase werd bij de voor de praktijk aanbevolen doseringen van aldicarb, oxamyl en ethoprofos niet beïnvloed.

Uit de laboratoriumproeven zou dus af te leiden zijn dat men in met nematiciden behandelde velden eerder een afname in aantasting door R.

solani kan verwachten dan een toename. Toch wordt in veldproeven een toename in het optreden van R. solani gevonden. Daarom wordt rekening gehouden met eensterk effect van de nematiciden op de mycofage bodemfauna. De meest frequent aangetroffen nematoden in het veld waren Aphelenchus avenae en Aphelenchoides spp.. Deze nematoden konden snel vermeerderd worden op mycelium van R. solani in petrischalen. In de rhizosfeer van planten waarvan het loof verwijderd was, namen de aantallen A. avenae zeer snel toe. Buiten de rhizosfeer waren de populatiedichtheden van A. avenae en Aphelenchoides spp. laag. Aangenomen werd dat de snelle toename van A. avenae het gevolg was van een opbloei van de microflora in de rhizosfeer van afstervende wortels. Aldicarb, oxamyl en ethoprofos vertraagden deze toename aanzienlijk.

De granulaire nematiciden bleken nauwelijks een effect op de stengelaantasting en delakschurftbezetting te hebben in een veld dat het voorafgaande najaar met dichloorpropeen gefumigeerd was. Kort na het poten kunnen metabolieten welke na het fumigeren nog in de grond aanwezig waren nog een toxisch effect op R. solani gehad hebben. In het gefumigerde veld bleek de bodemfauna bijna geëlimineerd te zijn. Later in het seizoen bleek de populatiedichtheid van A. avenae veel hoger te zijn in de rhizosfeer van planten in het gefumigeerde veld dan in het niet gefumigeerde veld. Hoewel ook in het gefumigeerde veld de granulaire nematiciden de toename van A. avenae sterk afremden, waren de aantallen in de rhizosfeer nog dermate hoog dat er nog een aanzienlijke onderdrukking van R. solani verwacht kon worden. In de met nematiciden behandelde veldjes werd daarom geen verhoogde aantasting waargenomen.

Aardappelstengels bedekt met mycelium van R. solani, werden gesimuleerd met vlasstrootjes welke in het laboratorium door de schimmel gekoloniserd waren. Nadat deze strootjes in het veld waren begraven, namen de mycofage nematoden snel in aantallen toe. In grond welke met aldicarb behandeld was, was de kolonisatie door deze nematoden gering. Ook deze proeven toonden aan dat de begrazing van mycelium en infectiestructuren van R. solani door mycofage nematoden in met nematiciden behandelde grond sterk gereduceerd zal zijn.

Om onderscheid te kunnen maken tussen de rol van mycofage nematoden en mycofage microarthropoden in de onderdrukking van R. solani werd in één veldproef het insecticide lindaan toegepast. Lindaan vehoogde de

stengelaantasting en lakschurftbezetting in vrijwel dezelfde mate als aldicarb. De nematoden populatie werd er niet door beïnvloed. Dit betekent dat in ieder geval in dit veld, mycofage microarthropoden waarschijnlijk een belangrijke rol spelen bij de begrazing van R. solani. Aldicarb, ethoprofos en lindaan bleken de populatiedichtheden van de meeste soorten springstaarten en mijten sterk te verlagen in aardappelvelden. De meest aangetroffen soorten springstaarten waren Tullbergia krausbaueri, Isotoma notabilis en Folsomia fimetaria en de meest aangetroffen mijten waren Pygmephorus sellnicki, Pygmephorus blumentritti, Coccotydeus sp. en Histiosoma litorale. De aantallen van de Pygmephorus spp. waren niet gereduceerd in met aldicarb en ethoprofos behandelde grond. De meest talrijke springstaart T. krausbaueri weinig opgenomen na toepassing van aldicarb, maar wel in met ethoprofos en lindaan behandelde velden.

T. krausbaueri, F. fimetaria en H. litorale konden in het laboratorium vermeerderd worden op petrischalen welke overgroeid waren met R. solani of in cultures van R. solani in gesteriliseerde grond. Pygmephorus spp. en Coccotydeus sp. konden niet vermeerderd worden op deze wijze, terwijl I. notabilis niet levend uit het veld geïsoleerd werd. In laboratorium experimenten waarbij microarthropoden aan met R. solani geïnfecteerde grond werden toegediend, bleken T. krausbaueri en F. fimetaria een sterke reductie van de stengelaantasting van aardappelen door R. solani te veroorzaken. De laagste aantallen van T. krausbaueri en F. fimetaria waarmee de ziekte significant gereduceerd werd waren respectievelijk 9800 en 430 springstaarten per liter grond. H. litorale werd in een mengsel met de mijt Rhizoglyphus robini in de grond gebracht, maar kon drie weken na inoculatie niet meer teruggevonden worden. In soortgelijke inoculatie experimenten bleek A. avenae bij een populatie-dichtheid van 500 nematoden per liter reeds een significante reductie van de stengelaantasting te veroorzaken. Een in cultuur gebrachte Aphelenchoides soort had geen effect op de stengelaantasting. Bij ca 20 °C was de reproductiesnelheid van nematoden één tot enkele dagen, terwijl dit voor de meeste mycofage springstaarten en mijten langer was.

Uit de laboratoriumproeven komt naar voren dat A. avenae en F. fimetaria tot de belangrijkste soorten gerekend moeten worden voor de onderdrukking van R. solani in het veld. Een waarschijnlijk geringere

bijdrage kan verwacht worden van T. krausbaueri en andere mycofage soorten welke in lagere aantallen in de bodem werden aangetroffen. Het is zeer goed mogelijk dat er soorten zijn, die in het laboratorium niet op mycelium van R. solani in kweek gebracht werden, maar bij lage dichtheden wel een significante reductie van de ziekte veroorzaken.

Alle pesticiden die de populatiedichtheid van de mycofage bodemfauna reduceren, zullen daardoor aan bodemschimmels, zoals R. solani gelegenheid geven zich beter te ontwikkelen. De verhoogde aantasting van aardappelen in met granulaire nematiciden behandelde velden laat zich dus het best verklaren door een verminderde begrazing van het pathogeen door de mycofage bodemfauna.

CURRICULUM VITAE

Tjaart Writser Hofman werd geboren op 1 november 1957 te Groningen. In 1977 behaalde hij het diploma Atheneum B aan de Rijksscholengemeenschap te Appingedam, waarna hij aan de Landbouwuniversiteit te Wageningen planteziektenkunde studeerde. In november 1984 behaalde hij het ingenieursdiploma met als hoofdvakken Fytopathologie en Landbouwplantenteelt en als bijvak Nematologie. Van november 1984 tot november 1987 verrichtte hij als wetenschappelijk assistent bij de vakgroepen Fytopathologie, Nematologie en Landbouwplantenteelt en Graslandkunde het onderzoek beschreven in dit proefschrift. Sinds november 1987 is hij werkzaam op de afdeling landbouwkundig onderzoek en marktontwikkeling van Duphar B.V. te 's-Graveland.