Control of Rhizoctonia stem and stolon canker of potato by harvest methods and enhancing mycophagous soil mesofauna

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Control of Rhizoctonia stem and stolon canker of potato by harvest methods and enhancing mycophagous soil mesofauna

M. Lootsma

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STELLINGEN

1. Lage bodemtemperaturen en droge bodemomstandigheden in het voorjaar remmen niet het onderdrukkend effect van de nematode *Aphelenchus avenae* en de springstaart *Folsomia fimetaria* op de stengelaantasting door *Rhizoctonia solani* bij aardappel.

   Dit proefschrift

2. Het onderdrukkend effect van *Folsomia fimetaria* op de stengelaantasting door *Rhizoctonia solani* bij aardappel is afhankelijk van de aanwezigheid van alternatieve voedselbronnen.

   Dit proefschrift

3. Organische bemesting in het voorafgaande jaar kan de schimmeletende bodemfauna zodanig stimuleren dat de stengelaantasting door *Rhizoctonia solani* bij aardappel wordt verlaagd.

   Dit proefschrift

4. Bij de biologische bestrijding van bodemziekten is het introduceren van antagonisten minder natuurlijk dan het scheppen van optimale omstandigheden voor reeds in de bodem aanwezige antagonisten.

5. Verhoging van de 'employability' van werknemers staat op gespannen voet met het voornemen van de regering om oudere werknemers de kans te geven langer door te werken.

6. De plannen voor de invoering van het studiehuis in het voortgezet onderwijs gaan ten onrechte uit van de veronderstelling dat de leerlingen gemotiveerd zijn.

7. Natuurbeheer is een contradictio in terminis.

8. De benaming 'proefschrift' veronderstelt ten onrechte dat het gaat om een eerste publicatie, vaak gaat het om de laatste.

   Driek van Wissen. Groot verkeerde-woordenboek der Nederlandse taal; BZZTôH, 's Gravenhage, 1996.

9. Garagehouders dienen de aanleg van hoge verkeersdrempels financieel te ondersteunen.

10. Zowel de bevalling van een proefschrift als die van een baby vindt zelden plaats op de uitgerekende datum.

Stellingen bij het proefschrift van M. Lootsma: 'Control of Rhizoctonia stem and stolon canker of potato by harvest methods and enhancing mycophagous soil mesofauna'.

Wageningen, 7 november 1997.

Abstract

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Rhizoctonia solani (AG-3) is a soil-borne plant pathogen that causes canker on potato stems and stolons, resulting in a reduced quantity and quality of the tuber yield. Two approaches for non-chemical control of stem and stolon canker in potato, caused by soil-borne inoculum, were investigated.

Two field experiments were conducted to investigate whether harvest methods of potato affect soil infestation with *R. solani*. Soil infestation was estimated on the basis of stem infections of potato in the subsequent year. Immature-crop-harvesting lowered the disease severity in the next crop compared with haulm pulling and chemical haulm killing. However, this harvest method was only successfull in controlling the disease when the formation of sclerotia did not start before harvest and the crop debris was incorporated into the soil with a rotary hoe.

Control of Rhizoctonia stem and stolon infections by mycophagous soil animals was investigated in experiments under controlled conditions (growth chambers) and in field experiments. The mycophagous soil mesofauna was equally effective in reducing of stem infections at 10 and 15 °C, and they were effective over a broad range of soil moistures. Under controlled conditions, adding dried fresh rape material to the soil enhanced the populations of the springtail *Folsomia fimetaria* and the nematode *Aphelenchus avenae*. *F. fimetaria* reduced stem canker under a broad range of conditions, but when rape was added to the soil at pH-KCl 6.2, its suppressive effect disappeared completely, probably due to the presence of alternative food sources.

In field experiments, oats grown as green manure crop or farmyard manure plus white mustard as green manure crop enhanced the populations of the mycophagous soil fauna and reduced the severity of Rhizoctonia stem and stolon canker. Oats especially increased the populations of mycophagous nematodes, whereas farmyard manure plus white mustard mainly enhanced the populations of mycophagous springtails.

Keywords: *Aphelenchus avenae*, farmyard manure, *Folsomia fimetaria*, green manure crop, harvest methods, mycophagous nematodes, potato, *Rhizoctonia solani*, soil infestation, soil mesofauna, *Solanum tuberosum*, springtails, stem canker.

"In tatebern sit by de hoale fan in grutte slange te boartsjen
en in lytse beuker lit de hân samar oer in nêst mei njirren gean."

(Bible in Frisian: Jesaja 11: 8)

Woord Vooraf

Tijdens een doctoraalonderzoek van mijn studie landbouwplantenteelt was ik al geboeid door de onderaardse wezentjes die de nederlandse akkers bevolken. Ik was dan ook erg blij toen mij de mogelijkheid werd geboden om als AIO dit onderzoek verder uit te werken naar een mogelijke inzet van deze beestjes bij een niet-chemische beheersing van de Rhizoctonia ziekte bij aardappel. Dit proefschrift bevat de neerslag van dat onderzoek. Op deze plaats wil ik graag een aantal mensen bedanken, die een aandeel hebben gehad bij de totstandkoming hiervan.

De direkte begeleiding was in handen van mijn co-promotor Klaas Scholte. Ik heb veel te danken aan zijn originele ideeën en grote kennis over alles wat met aardappelen te maken heeft. Klaas, je stond altijd voor me klaar en ik moet eerlijk zeggen, er zijn verschillende momenten geweest waarop ik dacht: dit wordt niks. Dankzij jouw onverzettelijk optimisme en geduld is het toch wat geworden. Ook mijn promotor Paul Struik wil ik graag hartelijk bedanken. Paul, ik heb grote bewondering voor de snelle, accurate en stimulerende wijze waarmee je werkte. Ook wil ik Jan Vos hartelijk bedanken voor zijn kritische commentaren op proefvoorstellen en manuscripten.

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Contents

1. General Introduction 1

2. Effects of soil disinfection and potato harvesting methods on stem infection by *Rhizoctonia solani* Kühn in the following year 7

3. Effects of the springtail *Folsomia fimetaria* and the nematode *Aphelenchus avenae* on *Rhizoctonia solani* stem infection of potato at temperatures of 10 and 15 °C 17

4. Effect of soil moisture content on the suppression of Rhizoctonia stem canker on potato by the nematode *Aphelenchus avenae* and the springtail *Folsomia fimetaria* 29

5. Effect of soil pH and amendments with dried fodder rape on mycophagous soil animals and Rhizoctonia stem canker of potato 41

6. Effect of farmyard manure and green manure crops on populations of mycophagous soil fauna and Rhizoctonia stem canker of potato 55

7. General Discussion 69

References 77

Summary 83

Samenvatting 85

Curriculum vitae 89

Chapter 1

General Introduction

Chapter 1

General Introduction

EFFECTS OF *RHIZOCTONIA SOLANI* ON THE POTATO CROP

Rhizoctonia solani AG-3 is an important pathogen in fields where potato is frequently grown. The fungus survives in the soil by sclerotia or monoloid cells which are formed on plant debris. Sclerotia are also formed on progeny tubers. After planting of seed tubers, plants can be infected either by soil-borne or by tuber-borne inoculum. Subterranean stem parts, stolons, and young tubers can become infected by the pathogen. Severe infections of stems lead to delayed and irregular emergence and fewer stems per plant (Scholte, 1989). Stolon pruning and infections of young tubers cause malformation of tubers, and size, numbers and skin colour of tubers are also adversely affected. Thus, severe plant infections cause lower total tuber yields and inferior tuber quality, resulting in a strong decrease in marketable yield of ware potato. Moreover, at the end of the growing season sclerotia may be formed on progeny tubers (black scurf) which is unwanted in seed tuber production.

PRESENT CONTROL METHODS

Both stem and stolon canker (further indicated as "stem canker") and black scurf formation can be controlled chemically. However, due to Dutch legislation the use of pesticides has to be reduced drastically and non-chemical control methods need to be developed. To prevent black scurf in seed tuber production, an effective non-chemical control method has been developed based on green-crop-harvesting (Mulder *et al.*, 1992). Harvesting methods affect the formation of sclerotia on tubers. However, whether they also affect the formation of sclerotia on plant debris has not been investigated. Harvesting methods could contribute to reducing *R. solani* soil infestation after growing a potato crop.

The reduction of black scurf formation by green-crop-harvesting can be enhanced by the application of the hyperparasite *Verticillium biguttatum* (Mulder *et al.*, 1992). However,

application of *V. biguttatum* in early spring on seed tubers does not effectively reduce stem canker. The efficacy of the hyperparasite is extremely low at temperatures <15 °C, whereas *R. solani* develops well at temperatures ≤ 10 °C (Van den Boogert & Jager, 1984). Until now, other antagonistic fungi or bacteria have not shown promising in controlling Rhizoctonia stem canker.

Reducing the frequency of potato in crop rotations could be an effective method for control of Rhizoctonia stem infection. *R. solani* AG-3 has a very narrow host range (Carling *et al.*, 1986; Carling & Leiner, 1986). The fungus shows a pathogenic relationship with potato only and possibly an epiphytic relationship with other plant species. Although the fungus can survive for at least five years in the soil, stem infections are acceptably low in crop rotations with less than 20% potato (Scholte, 1992). However, such low frequencies of potato are not economically attractive in the Netherlands.

As stated, fungal antagonists are not very promising in controlling Rhizoctonia stem canker. However, Hofman (1988) showed that the mycophagous mesofauna can also reduce plant infections. He found that the springtails *Folsomia fimetaria* and *Tullbergia krausbaueri* and the nematode *Aphelenchus avenae* were very suppressive. Moreover, these organisms can also be found in rotations with high frequencies of potato (Scholte, 1987). These organisms are indigenous in a broad range of soil types, as indicated by Scholte (1987) and Hofman (1988). Reduction of populations of mycophagous soil animals by granular insecticides/nematicides (aldicarb, oxamyl and ethoprophos) or by lindane increased stem canker both on calcareous marine clay soils and sandy soils.

OBJECTIVES AND OUTLINE OF THIS THESIS

At present, the non-chemical methods available to control stem canker on potato are inadequate. The objective of this thesis is to explore the potential of new bio-ecological techniques for non-chemical control of stem canker of potato caused by soil-borne inoculum of *R. solani*. Two approaches have been investigated:

a. farm hygiene to reduce the inoculum level in the soil;

b. stimulation of the suppressing potential of the soil mesofauna.

In Chapter 2, the effects of various harvesting techniques for potato on soil infestation with *R. solani* are analyzed. The subsequent chapters deal with the question whether it is possible to

make use of the suppressing effect of the mycophagous soil fauna on stem canker. In Chapters 3, 4 and 5, the effects of abiotic and biotic factors on the suppressive capacity of two important mycophagous soil animals, *F. fimetaria* and *A. avenae*, are described. Bio-assays under controlled conditions were used to investigate the effect of soil temperature (Chapter 3), soil moisture (Chapter 4) and addition of organic amendments at two pH levels (Chapter 5) on the suppression of stem canker by these two mycophagous organisms. Chapter 6 deals with the effect of various organic amendments on the mycophagous soil fauna and on Rhizoctonia stem canker under field conditions. The general discussion (Chapter 7) reviews the progress made in this research programme in the light of the original objectives and discusses the potential of these non-chemical bio-ecological approaches.

Chapter 2

Effects of soil disinfection and potato harvesting methods on stem infection by *Rhizoctonia solani* Kühn in the following year

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Chapter 2

Effects of soil disinfection and potato harvesting methods on stem infection by *Rhizoctonia solani* Kühn in the following year

M. LOOTSMA and K. SCHOLTE

SUMMARY

A two year field experiment was conducted twice to assess effects of chemical soil disinfection at planting and methods of harvesting potatoes on stem infection with *Rhizoctonia solani* in the subsequent year. In the first year of the experiments seven methods, including one with soil disinfection at planting, were applied in August. In the following year, *R. solani* stem and stolon infection (disease severity) on potato plants were assessed in June.

Soil treatment at planting with pencycuron resulted in lowest disease severity in the following year. Compared with chemical haulm killing and haulm pulling, immature-crop-harvesting also resulted in a lower disease severity, but only when black scurf was scarce on tubers at harvest in the preceding year.

INTRODUCTION

Rhizoctonia solani (AG-3) is a soil-borne plant pathogen that causes canker on potato stems and stolons and black scurf on tubers. Severe canker results in delayed emergence, fewer emerged stems, lower tuber yield, a high proportion of small, partly green and misshapen tubers with a lower dry matter content and also an increased proportion of very large tubers (Van Emden, 1958; Hide *et al.*, 1985, 1989; Scholte, 1989).

The infection of potato plants in early spring can arise from tuber-borne or soil-borne inoculum. Especially in crop rotations with a high frequency of potato (Hide & Read, 1991; Scholte, 1987), soil-borne *Rhizoctonia* can significantly reduce the yield and quality of potato

tubers (Frank, 1975; Scholte, 1989; Simons, 1990). *R. solani* AG-3 has a small host range (Carling *et al.*, 1986), but potato is a good host and therefore the build-up of soil inoculum by growing potatoes may play an important role in its population dynamics. Controlling this build-up of soil inoculum can contribute to the non-chemical control of soil-borne stem canker. Boosalis & Scharen (1959) and Van den Boogert & Velvis (1992) found that surviving structures of *R. solani* are found mainly on and in plant debris.

Sclerotia (black scurf) form on progeny tubers and other underground plant parts mostly at the end of the growing season. The level of black scurf on tubers can be decreased by soil disinfection with fungicides. For example, soil treatment with pencycuron or tolclofos-methyl at the recommended doses at planting is very effective (Mulder & Roosjen, 1982; Jager *et al.*, 1991). Also harvesting methods that are used in potato seed production in the Netherlands can affect the level of black scurf. Chemical haulm destruction enhances black scurf more than haulm pulling (Reestman & Scheepers, 1955; Van Emden *et al.*, 1966; Mulder & Roosjen, 1982; Bouman *et al.*, 1983; Dijst, 1985). Green-crop-harvesting (harvesting the immature crop by machinery and replacing the tubers into the soil for curing and final harvesting 2-4 weeks later) often results in low levels of black scurf (Mulder *et al.*, 1992). This is especially the case with immature-crop-harvesting (pulling haulm and collecting the tubers by hand) (Van Emden *et al.*, 1966). However, whether these haulm destruction methods and soil disinfection also affect the amounts of soil inoculum and disease severity in the subsequent years has not been investigated. Therefore, experiments were conducted to study the effects of such treatments on stem and stolon canker in the following season.

MATERIALS AND METHODS

Experimental set up

Two-year field experiments were carried out in 1991 - 1992 (Experiment 1) and in 1993-1994 (Experiment 2). The experiments were sited in Achterberg, close to Wageningen, on a sandy soil (fractions: 3.2% <2 μm, 4.7% 2 - 50 μm and 92.2% 16-2000 μm) with a pH-KCl of 5.2 and an organic matter content of 3.9 %. In Experiment 1 the preceding crops were potato (c. Prominent) in 1989 and sugar beet (c. Univers) in 1990 and in Experiment 2 potato (c. Prominent) in 1991 and maize (c. Brutus) in 1992. In both experiments potatoes were grown in two consecutive years.

N, P, K and Mg fertilizers were applied each year at standard recommended quantities.

Ridges were prepared directly after planting in both experiments. Weeds and late blight were controlled chemically. When necessary, fields were irrigated during the growing season.
In April of Year 1 pre-sprouted seed tubers of cv. Santé (Experiment 1) or Spunta (Experiment 2), severely infested with black scurf, were mechanically planted 30 cm apart at a row distance of 75 cm. Plots were 6 x 6 m^2, containing 8 rows of potato. The four inner rows were used for observations. The experiments were laid out as randomized complete block designs with seven treatments and six replications.

The treatments (Table 1) were applied in August of Year 1 except the soil disinfection in Treatment 1. In Treatment 1 soil disinfection with pencycuron (Monceren, 25% a.i., Bayer Nederland bv, 25 kg/ha of the trade product), applied one day before planting in April, was combined with chemical haulm destruction. For chemical haulm destruction in Treatments 1 and 2, DNOC (Luxan DNOC-oil, 200 g a.i./l, 25 l/ha) was applied four weeks before harvesting. Haulm pulling (Treatment 3) was done by hand four weeks before harvesting the tubers. Potato tubers of Treatment 4 were harvested after complete natural plant senescence. Immature-crop-harvesting was performed by lifting the potato plants by hand on the same dates as chemical haulm killing and haulm pulling in Treatments 1 to 3. The remaining plant debris was removed from the field (Treatment 5), left on the field (Treatment 6) or chopped into pieces of about 30 cm length and incorporated into the soil one day after tuber harvesting using a rotary hoe at slow speed (Treatment 7). Black scurf severity on tubers was assessed after harvest. Potatoes were also grown in Year 2 of the experiments. Before planting, seed tubers (size 35-40 mm) of cv. Element visibly free from black scurf were disinfected by immersing them in a solution of

Table 1. Treatments in Experiments 1 and 2.

Haulm treatment	Tuber harvest[a]	Plant debris
1 Pencycuron[b] + chemical haulm destruction	28	left on soil surface
2 Chemical haulm destruction	28	left on soil surface
3 Haulm pulling	28	left on soil surface
4 Natural senescence	63	left on soil surface
5 Immature-crop-harvest	0	removed from the field
6 Immature-crop-harvest	0	left on soil surface
7 Immature-crop-harvest	0	incorporated into the soil

[a]Days after immature-crop-harvest in August.

[b]Pencycuron incorporated into the soil one day before planting in April.

validamycine (Solacol, AAgrunol, 30 g/l a.i., 3% solution of the trade product). In April the seed tubers were planted by hand at a spacing of 20 x 75 cm to achieve a high stem density. Stem and stolon canker caused by *R. solani* were assessed in June.

Determination of black scurf on tubers in Year 1

At harvest in Year 1 of both experiments, samples of 100-150 tubers were taken from the four inner rows of each plot to assess the effect of treatments on black scurf. Disease severity on each tuber was recorded using 5 classes (Dijst, 1985) and converted to a black scurf-index (BI = 0-100) using the following formula:

$$BI = 100 \times (0 \times n_0 + 1/4 \times n_1 + 2/4 \times n_2 + 3/4 \times n_3 + n_4) / n_{total}$$

where n = the number of tubers in each category 0-4.

Determination of stem and stolon infection in Year 2

In both experiments, stem and stolon infection caused by *R. solani* were assessed on June 15 . Forty plants with on average three main stems were harvested per plot. Stem infection was recorded on each stem and classified as follows:

0 = no lesions,
1 = some small lesions scattered over the stem,
2 = moderate number of small lesions not covering >25% of the stem,
3 = major lesions covering >25% of the stem, but no girdling of the stem,
4 = major lesion including girdling of the stem,
5 = severe lesions killing the stem.

Stolon infection was recorded per plant using a classification from 0 - 5 (analogous to the classes of stem canker). Stem and stolon infection were each converted into a disease index (DI = 0 -100), using the following formula:

$$DI = 100 \times (0 \times n_0 + 1/5 \times n_1 + 2/5 \times n_2 + 3/5 \times n_3 + 4/5 \times n_4 + n_5) / n_{total}$$

where n = the number in each category 0-5.

Statistical analysis

Data of both experiments were combined in a split-plot analysis in time using ANOVA. Least significant differences between treatments were calculated using the LSD-test.

RESULTS

Black scurf severity in Year 1

Black scurf severity was higher in Experiment 2 (1993) than in Experiment 1 (1991), except for the pencycuron treatment (Table 2). Effects of the various treatments on black scurf severity on tubers were pronounced and lowest severities were obtained with immature-crop-harvest or when the soil was treated with pencycuron at planting. Highest incidence of black scurf occurred with chemical haulm killing without soil disinfection, whereas haulm pulling and natural senescence showed intermediate levels.

Stem and stolon canker in Year 2

There was a close correlation between stem canker and stolon canker (Fig.1). The regression coefficients (Exp. 1 : 1.07, SE= 0.08, 40 df; Exp. 2: 1.28, SE= 0.10, 40 df) of both experiments did not differ significantly. Table 3 presents means of the stem and stolon canker indices. Stem and stolon canker were lower in Experiment 1 than in Experiment 2, except for the pencycuron treatment and haulm pulling. In both experiments, soil treatment with pencycuron in Year 1 resulted in relatively low infection levels in Year 2, and especially in Experiment 2. In Experiment 1, immature-crop-harvesting tended to give less disease than haulm pulling and chemical haulm destruction, especially when plant debris was incorporated into the soil, but this effect did not occur in Experiment 2.

Table 2. Effect of soil disinfection with pencycuron at planting and harvest methods in Year 1 on black scurf index (0 - 100) on progeny tubers in Year 1.

	Treatment	Experiment 1	Experiment 2
1	Pencycuron + Chemical haulm killing	29	26
2	Chemical haulm killing	41	65
3	Haulm pulling	34	44
4	Natural senescence	31	58
5-7	Immature-crop-harvest	7	26

LSD for comparing means is 5.6 ($P = 0.05$).

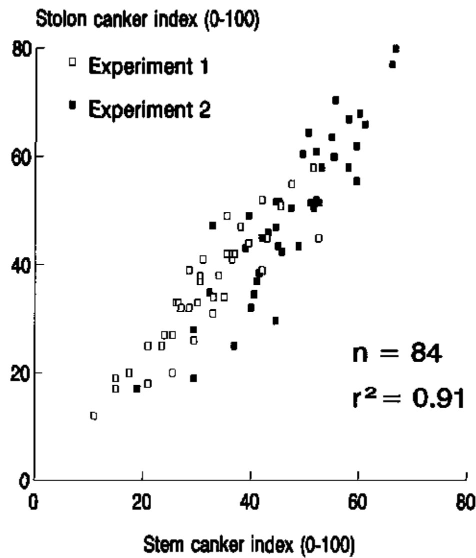

Fig.1. Relation between stem and stolon canker index.

Table 3. Effect of soil disinfection with pencycuron at planting and harvest methods in Year 1 on stem and stolon canker (disease-index 0 - 100) in Year 2.

Treatment		Experiment 1	Experiment 2
1	Pencycuron + chemical haulm killing	28	33
2	Chemical haulm killing	40	56
3	Haulm pulling	45	52
4	Natural senescence	39	48
	Immature-crop-harvest:		
5	- debris removed	32	51
6	- debris left on the soil	34	49
7	- debris incorporated into the soil	22	51

LSD for comparing means is 8.4 ($P = 0.05$).

DISCUSSION

Black scurf in Year 1

In these experiments the effect of soil disinfection and various harvest methods on black scurf severity on progeny tubers are in agreement with results from other researchers. Application of pencycuron resulted in equal levels of black scurf in both experiments, although the mean level of black scurf was higher in Experiment 2 than in Experiment 1. It is very unlikely that this difference in black scurf level between the Experiments was caused by the difference in preceding crop (sugar beet in Exp.1 and maize in Exp. 2) (Scholte, 1992). We surmise that also the use of seed tubers from different cultivars (Santé in Exp. 1 and Spunta in Exp. 2) to introduce *R. solani* in the fields did not affect soil infestation (Buhr, 1989). Probably, the higher level of black scurf in Experiment 2 could be attributed to lower temperatures and wetter conditions in the soil. For example, mean soil temperature at 10 cm depth and precipitation in the period 30 days before to 30 days after immature-crop-harvesting were 20.9 or 17.3 °C and 48 or 193 mm rainfall for Experiments 1 and 2, respectively.

Immature-crop-harvesting was less effective in Experiment 2 because formation of sclerotia had already started by the date of immature-crop-harvesting.

Disease severity in Year 2

It may be supposed that differences in plant infection in Year 2 between treatments are caused by different levels of soil infestation with *R. solani* because the plant debris (which is the main source of soil inoculum) was returned to the field (except Treatment 5) and all other factors possibly interacting with the disease level, like soil fertility, soil moisture and soil temperature did not differ between the treatments. Tuber-borne infection in Year 2 was excluded because visibly sclerotia-free seed tubers were disinfected with validamycine which had been proven to be very effective in controlling *R. solani* in earlier experiments (K. Scholte, personal observations). Only the antagonistic soil flora and fauna could possibly interact with disease levels in Year 2.

Soil disinfection with pencycuron appeared to have a significant after-effect on the disease severity in the following year. Pencycuron is very effective in inhibiting the growth of *R. solani* (Kataria & Gisi, 1989; Kataria *et al.*, 1989). Thus it may be expected that at the end of the growing season in Year 1, low levels of *Rhizoctonia* were present in the soil, resulting in the formation of low levels of resting structures. This was demonstrated by the black scurf index in

Year 1. Probably also low levels of survival structures are formed on plant debris remaining in the soil.

In Experiment 1 immature-crop-harvesting, especially when combined with incorporation of plant debris into the soil, resulted in a lower disease index in Year 2, compared with the other harvesting methods. This can be caused by a lower soil infestation. Dijst (1990) showed that volatile and unstable exudates from underground plant parts stimulate the formation of resting structures. Sufficient aeration of the soil would reduce the accumulation of these components (Dijst *et al*, 1986). Using the rotary hoe clearly resulted in a higher aeration of the soil. In Experiment 2 no reducing effect of immature-crop-harvesting was observed. Formation of black scurf had already reached a considerable level at immature-crop-harvesting date (Table 2), and this fact may have obscured effects. It may be expected that only at lower soil infestation levels there will be a (linear) relationship between level of soil infestation and disease severity.

It can be concluded that soil disinfection with pencycuron results in a lower soil infestation level in the subsequent year, but the effect of harvest methods seems to depend on soil infestation level at immature harvest time.

Chapter 3

Effects of the springtail *Folsomia fimetaria* and the nematode *Aphelenchus avenae* on *Rhizoctonia solani* stem infection of potato at temperatures of 10 and 15 °C

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Chapter 3

Effects of the springtail *Folsomia fimetaria* and the nematode *Aphelenchus avenae* on *Rhizoctonia solani* stem infection of potato at temperatures of 10 and 15 °C

M. LOOTSMA and K. SCHOLTE

SUMMARY

Effects of mycophagous soil mesofauna on *Rhizoctonia solani* infection of potato stems were investigated in two experiments carried out in growth chambers at 10 and 15 °C. The springtail *Folsomia fimetaria* and the nematode *Aphelenchus avenae* were selected as organisms suppressive to *R. solani*.

Reduction in Rhizoctonia stem infection by mycophagous soil mesofauna was equally effective at 10 and 15 °C. *F. fimetaria* tended to be more effective than *A. avenae*, but the best control was obtained when both organisms were present in the soil at high densities.

INTRODUCTION

Rhizoctonia solani (AG-3) is a serious pathogen of potato, especially of young sprouts of seed tubers, which can be severely infected by the fungus. In the Netherlands, potatoes are planted from approximately mid-March until early May, when soil temperatures range from 8 - 13 °C. At this temperature range, infection of potato by *R. solani* is greatest (Bolkan *et al.*,1974; Hide & Firmager, 1989; Carling & Leiner, 1990).

R. solani can be suppressed by fungal antagonists or by mycophagous soil fauna (Hofman, 1988; Hofman *et al.*, 1990). Most fungal antagonists of *R. solani* need relatively high soil temperatures (>18°C) to be effective (Boosalis, 1956; Beagle-Ristaino & Papavizas, 1985). *Verticillium biguttatum,* an important fungal antagonist of *R. solani*, also requires soil

temperatures >13°C for effective suppression of *R. solani* (Van den Boogert & Saat, 1991). Most investigations on the suppressive potential of mycophagous springtails and nematodes on *R. solani* and other root rot diseases have been carried out at high soil temperatures (20 - 35 °C) (Klink & Barker, 1968; Barnes *et al.*, 1981; Caubel *et al.*, 1981; Curl, 1979; Ulber, 1983). However, Bollen *et al.* (1990) demonstrated that the springtail *Folsomia fimetaria* and the nematode *Aphelenchus avenae* were suppressive to Rhizoctonia stem canker of potato at 15 °C.

We have studied the effects of soil mesofauna on Rhizoctonia stem infection of potato at 10 and 15 °C. This temperature range parallels that occurring in the field from planting to emergence of the potato crop in the Netherlands. A new test procedure was developed, with a view to providing time for the *R. solani* inoculum and the mycophagous soil fauna to adapt to the environmental conditions of the experiment before the infection capacity of *R. solani* on potato sprouts was measured. The springtail *Folsomia fimetaria* and the nematode *Aphelenchus avenae* were used as test organisms, because they are important representatives of the mycophagous fauna in agricultural soils (Hofman *et al.*, 1990). This report describes the test procedure and the first results obtained with it.

MATERIALS AND METHODS

Preparation of *R. solani* inoculum

In two experiments a pathogenic strain of *R. solani* AG-3, isolated from potato and stored on potato dextrose agar (PDA), was used. For mass production of sclerotia, the fungus was transferred to a medium containing 15 g/l malt extract, 12 g/l agar, 5 g/l yeast extract and 1 g/l peptone. After three weeks at 20 °C the sclerotial mats were fragmented in water with a blender (Braun minipimer, equipped with a 3 cm blade), sieved, dried and sieved again. Sclerotia of 0.4 - 1 mm diameter were used to infest soil.

Rearing of *F. fimetaria*

F. fimetaria was obtained from soil collected from a potato field, using a modified computerized Petersen extraction apparatus (Petersen, 1978). The animals were maintained in plastic Petri dishes on a culture of *Alternaria porri* on PDA and transferred every 8 weeks to fresh cultures of *A. porri*. *A. porri* is not pathogenic to potato and does not influence infection by *R. solani* (Hofman, 1988).

Approximately 7 weeks before the beginning of an experiment, mass cultures were started to obtain high numbers of *F. fimetaria*. Potting soil (60 % w/w organic matter) was mixed with wheat meal (5 % w/w) and autoclaved for 20 minutes at 120 °C in 500 ml Erlenmeyer flasks. The soil-meal mixture was then inoculated with *A. porri* and incubated at 20 °C for 2 - 3 weeks. Flasks were then each amended with 100 to 500 animals, and after 3 - 4 weeks at 20 °C a mass culture with a very high population of *F. fimetaria* was obtained. Three days before starting experiments the contents of all Erlenmeyer flasks were gently but thoroughly mixed; six subsamples of c. 40 g mixture were weighed accurately and transferred to the extraction apparatus. After two days incubation with temperature slowly increasing from 20 °C to 40 °C, the subsamples were counted under a stereo microscope (magnification x 60) and the population of springtails in the mass culture was estimated.

Rearing of *A. avenae*

Nematodes were extracted from soil collected from a potato field using an Oostenbrink elutriator (Oostenbrink, 1960). Maintenance and mass rearing of *A. avenae* were carried out in a similar way as for *F. fimetaria*. Populations of *A. avenae* were determined by transferring c. 30 g mixture to a filter paper contained within a 0.6 mm mesh sieve. This sieve was placed on a metal container with 100 ml tap water. After 24 hours the nematodes had passed through the sieve into the water below. Nematode numbers in two 5 ml subsamples were counted under a stereo dissecting microscope (magnification x 100).

Test procedure

To test the effects of the mycophagous soil fauna on Rhizoctonia infection of potato sprouts a special set-up was used. Twenty-eight litre rectangular plastic containers slanting from the top to the bottom were used, with inner dimensions at the top of 40 x 33 cm and at the bottom of 36 x 29 cm; the height was 24 cm. Each experimental unit consisted of two containers. In the base of Container 1 there were twenty 2.5 cm holes, 6.5 cm apart, in a grid of 5 x 4. This container was filled with a 15 cm layer of a mixture (v/v) of 75% pure quartz sand (fractions: 0.8% <50 μm, 89.4% 50 - 2000 μm and 9.8% 2000 - 4000 μm) and 25% enriched peat soil (60% peat + 40 % silty clay (w/w) + nutrients, 57% organic matter). The peat soil had been pasteurized at 70 °C for two hours, but the sand had not been treated. The soil mixture (pH-KCl 5.5, 5.0% organic matter) was amended with sclerotia of *R. solani* and, depending on the treatment, with different populations of mycophagous soil fauna. Mass cultures, containing the desired numbers

of springtails or nematodes according to the treatment, were added to the soil in Container 1 in two narrow horizontal layers at 5 and 10 cm depth. The optimum density of *R. solani* sclerotia and methods of introduction of *R. solani*, *F. fimetaria* and *A. avenae* were determined in preliminary experiments. These showed that the animals added became evenly distributed through the soil within two weeks. Granulated baker's yeast (Baukje Bakproducten B.V. Rijssen) was also added (3.5 g/l soil), as in preliminary experiments it was found that addition of yeast enhanced the build-up of the populations of the mycophagous soil fauna, without affecting Rhizoctonia stem infection. After inoculation, the containers were placed at 15 °C to allow the populations to stabilize. Two weeks after inoculation this container was placed in the second container (Container 2) without holes. At the bottom of Container 2 there was a layer of 6 cm moist pure sand in which 20 single-sprouted tubers of c. 30 mm diameter of potato cv. Element were buried in a 5 x 4 grid corresponding with the grid of holes in the base of Container 1. The soil layer in Container 1 made close contact with the tubers in Container 2. The seed tubers were visibly free from black scurf and had been disinfected against *R. solani* by immersion in a solution of validamycine (Solacol, AAgrunol, 30 g/l a.i., 3% solution of the trade product) for 10 seconds. The potato sprouts grew through the holes and into the soil layer of Container 1. The containers were placed in the dark in a growth chamber at temperatures according to the treatments. Soil moisture was maintained at pF 2.7 - 3.1 by daily wetting. However, in Experiment 1 from Day 1 to Day 14 soil was not wetted and therefore soil moisture decreased from pF 2.9 to lower moisture levels during this period. When the sprouts emerged from the soil layer the growth chamber was faintly illuminated (60 W/m^2) for 12 hours per day to limit etiolation. Four weeks after planting, when the majority of sprouts had emerged, sprouts were harvested and severity of *R. solani* was assessed.

Experiments

Two experiments were carried out in growth chambers: Experiment 1 from October 14 - November 25 1991 and Experiment 2 from February 24 - April 13 1992. In each experiment, one growth chamber was set at 10 °C and one at 15 °C. On Day 1, soil was infested with 20 sclerotia of *R. solani* per litre soil and thoroughly mixed using a concrete mixer. In Experiment 1, *F. fimetaria* and *A. avenae* were added at populations of 0, 250 or 1000 and 0, 1500 or 22500 per litre soil, respectively. In Experiment 2, these populations were 0, 240 or 720 and 0, 800 or 8300, respectively. The numbers of *A. avenae* were lower in Experiment 2 than in Experiment 1 due to a lower multiplication rate in the mass culture. The lower multiplication

rate of *A. avenae* caused a later start of the experiment than planned. Therefore, sclerotia of *R. solani* were stored for several weeks prior to use. Two weeks after inoculation, Container 1 was placed in Container 2, as described previously and placed in a growth chamber at 10 or 15 °C, depending on the treatment.

Each experiment consisted of 18 treatments (3 populations of *F. fimetaria* x 3 populations of *A. avenae* x 2 temperatures) with one container-set as an experimental unit. Within each growth chamber (temperature treatment) the nine *F. fimetaria* x *A. avenae* treatments were replicated in four blocks.

Records

The severity of stem infection caused by *R. solani* on the subterranean part of each potato stem was assessed 4 weeks after planting as follows:

0 = no lesions,
1 = some small lesions scattered over the stem,
2 = moderate number of small lesions covering 25% of the stem,
3 = major lesions covering 25% of the stem, but no girdling of the stem,
4 = major lesions, including girdling of the stem,
5 = lesions severe, stem death.

A disease-index (DI = 0 - 100) was calculated as follows:

$$DI = 100 \times (0 \times n_0 + 1/5 \times n_1 + 2/5 \times n_2 + 3/5 \times n_3 + 4/5 \times n_4 + n_5) / n_{total}$$

where n = the number in each category.

On Day 14, eight soil samples of 55 ml each were taken per container, using a 5 cm-diameter auger, to assess the population density of *A. avenae* and *F. fimetaria*. Four samples were transferred to the modified Petersen extractor for assessing the population of *F. fimetaria*. The other four samples were mixed and a subsample of 50 ml was used to determine the population of *A. avenae*.

Statistical analyses

Data of each experiment were analyzed in a split-plot analysis with growth chambers as main plots. Both growth chambers were the same make and age, and temperature conditions were controlled within ± 0.5 °C, so it may be assumed that effects on disease severity due to differences between growth chambers were negligible compared to the effects of the temperature treatments.

RESULTS

Experiment 1

In the absence of *F. fimetaria* or *A. avenae* the disease index was high and tended ($P<0.10$) to be higher at 10 °C than at 15 °C (Table 1). *F. fimetaria* decreased the disease index ($P<0.001$) at 10 °C but not at 15 °C. *A. avenae* decreased the disease index at 10 °C ($P<0.001$) and at 15 °C ($P<0.01$), but the decrease was highest at 10 °C ($P<0.05$).

The effect of *F. fimetaria* and *A. avenae* on the disease-index showed a synergistic interaction (Table 2). *F. fimetaria* and *A. avenae* had no significant effect when applied alone. However, in the presence of 250 *F. fimetaria* per litre soil, disease severity was reduced ($P<0.01$) by the highest population of *A. avenae*. A further reduction in disease severity occurred with 1000 *F. fimetaria* per litre soil in the presence of the highest population of *A. avenae* ($P<0.001$).

The numbers of *F. fimetaria* and *A. avenae* had increased considerably 14 days after inoculation (Table 3).

Experiment 2

Disease severity was lower than in Experiment 1 (Tables 1 and 4) and, in contrast to Experiment 1, was higher ($P<0.01$) at 15 than at 10 °C (Table 4). At both temperatures *F. fimetaria* was effective when applied alone, again in contrast to Experiment 1. *A. avenae* did not

Table 1. Effect of *F. fimetaria* (averaged over three *A. avenae* levels) and *A. avenae* (averaged over three *F. fimetaria* levels) on Rhizoctonia disease index (0-100) of potato stems at two soil temperatures. Experiment 1.

Temperature (°C)	*F. fimetaria* (numbers/l soil)			*A. avenae* (numbers/l soil)		
	0	250	1000	0	1500	22500
10	80	75	60	83	76	56
15	71	65	65	72	69	60
SED (47 d.f.)[a]		4.1			4.1	

The two-factor interactions Temperature * *F. fimetaria* ($P<0.05$) and Temperature * *A. avenae* ($P<0.05$) are significant.

[a]For comparisons of means at the same level of temperature.

Table 2. Effect of *F. fimetaria* and *A. avenae* on Rhizoctonia disease index (0-100) of potato stems; averaged over two soil temperatures. Experiment 1

F. fimetaria (numbers/l soil)	*A. avenae* (numbers/l soil)		
	0	1500	22500
0	77	75	73
250	78	72	60
1000	77	70	40
SED (47 d.f.)		5.0	

The interaction *F. fimetaria* * *A. avenae* is significant (P<0.001).

Table 3. Population development of *F. fimetaria* and *A. avenae* from Day 1 to Day 14. Experiment 1

Numbers/l soil inoculated		Numbers/l soil on Day 14	
F. fimetaria	*A. avenae*	*F. fimetaria*	*A. avenae*
0	0	0	0
0	1500	0	10700
0	22500	0	79000
250	0	1165	0
250	1500	1016	8000
250	22500	973	52300
1000	0	2933	0
1000	1500	4740	3390
1000	22500	2758	45950

Table 4. Effect of *F. fimetaria* (averaged over three *A. avenae* levels) and *A. avenae* (averaged over three *F. fimetaria* levels) on Rhizoctonia disease index (0-100) of potato stems at two soil temperatures. Experiment 2

Temperature (°C)	*F. fimetaria* (numbers/l soil)			*A. avenae* (numbers/l soil)		
	0	240	720	0	800	8300
10	37	20	12	25	22	22
15	51	28	23	41	31	30
SED (48 d.f.)[a]		5.5			5.5	

Main effects of Temperature (P<0.01) and *F. fimetaria* (P<0.001) are significant, whereas the effect of *A. avenae* is not significant.

[a]For comparisons of means at the same level of temperature.

Table 5. Effect of *F. fimetaria* and *A. avenae* on Rhizoctonia disease index (0-100) of potato stems; averaged over two soil temperatures. Experiment 1

F. fimetaria (numbers/l soil)	*A. avenae* (numbers/l soil)			
	0	800	8300	**Mean**
0	56	36	41	**44**
240	27	26	19	**24**
720	16	18	18	**17**
Mean	**33**	**27**	**27**	
SED (48 d.f.)[a]		3.9		

There is no significant interaction between *F. fimetaria* * *A. avenae*. Main effect of *F. fimetaria* is significant ($P<0.001$).
[a]For comparisons of means concerning the main effects for *F. fimetaria* or *A. avenae*.

Table 6. Population development of *F. fimetaria* and *A. avenae* from Day 1 to Day 14. Experiment 2

Numbers/l soil inoculated		Numbers/l soil on Day 14	
F. fimetaria	*A. avenae*	*F. fimetaria*	*A. avenae*
0	0	0	0
0	800	0	330
0	8300	0	6065
240	0	691	0
240	800	768	3030
240	8300	874	7250
720	0	1113	0
720	800	1208	1165
720	8300	1208	4750

significantly affect the disease severity (Table 4) and there was no apparent synergistic effect when both organisms were introduced together (Table 5), unlike in Experiment 1. Populations of *F. fimetaria* increased slightly during the first 14 days of the experiment. However, populations of *A. avenae* fluctuated considerably between treatments (Table 6).

DISCUSSION

The method used to investigate the suppressive potential of mycophagous soil mesofauna on *R. solani* appeared to be satisfactory but needs further improvement. Within experiments the variation of *R. solani* soil infestation was minimized to the extent that the antagonistic effects of the soil fauna were measurable. Although the same populations of sclerotia of *R. solani* were added to the soil in Experiments 1 and 2, sclerotia in Experiment 1 were applied immediately after production, whereas those used in Experiment 2 had been first stored for several weeks. Therefore, in the latter case it may be expected that sclerotia were less infectious, because the thin-walled hyphen that can be observed on the surface of young sclerotia die when stored under dry conditions at room temperature. This explains why in all treatments, except one, disease severity was lower in Experiment 2 than in Experiment 1.

Between Experiments 1 and 2 there was a considerable difference in the pattern of population development of the mycophagous soil animals from Day 1 to 14. In both experiments the populations of *F. fimetaria* had increased two weeks after introduction, though the multiplication rate was greater in Experiment 1 than in Experiment 2 (on average x3.9 and x2.4, respectively). However, the multiplication rate of *A. avenae* differed considerably between the experiments. Whereas in Experiment 1 numbers increased (on average x3.7) after introduction, numbers in Experiment 2 increased very little (on average x1.3) and fluctuated considerably between treatments. Also, the multiplication of the nematodes in the mass culture for Experiment 2 was rather low. There is not a satisfying explanation for this behaviour of *A. avenae*, but it has undoubtedly affected the results of Experiment 2. In this experiment, *A. avenae*, applied alone or combined with *F. fimetaria*, had no affect at all on the disease severity.

Hide & Firmager (1989) found that stem canker was equally severe at 10 and 15 °C. In our experiments the disease severity at 10 and 15 °C was not consistent between Experiments 1 and 2. Differences in soil moisture during Day 1 to 14 between the two experiments may have caused this discrepancy. A better standardization of environmental conditions during this period is needed and can be obtained by covering the containers with a plastic sheet to avoid desiccation in the first two weeks, as was done in experiments following these ones.

In Experiment 1 the inoculum potential of *R. solani* was presumably so high that the populations of *F. fimetaria* or *A. avenae*, when each was applied alone, were insufficient for control. Only when *F. fimetaria* was combined with the highest populations of *A. avenae* was the disease severity reduced. *R. solani* pressure from the soil was lower in Experiment 2 than in

Experiment 1 and therefore *F. fimetaria* when applied alone could effectively control the disease. The additive effect of *A. avenae* did not occur in Experiment 1, probably due to the much lower populations of the nematodes in this experiment. The degree of soil infestation with *R. solani* seems to be important. When inoculum is abundant, mycophagous animals probably fail to control *R. solani* due to lack of contact.

It is difficult to assess the populations of mycophagous soil organisms needed for effective control, especially since the degree of soil infestation with *R. solani* is likely to play an important role. Bollen *et al.* (1990) found that suppression of infection by *R. solani* occurred only at relatively high populations of *F. fimetaria*, when soil had been infested with relatively large quantities of fresh *R. solani* mycelium on wheat flour. Curl (1979) found that 1000 - 2000 springtails per kg soil were needed to control *R. solani* in cotton. Also, the interactions between mycophagous soil organisms affect the populations of soil organisms required for effective control. In our experiments only two organisms were used, whereas in field soil there are many more mycophagous species. Also the spatial pattern and population dynamics of mycophagous soil organisms are of importance (Hofman & s'Jacob, 1989). However, the fact that *R. solani* infection of potato stems increases when mycophagous soil fauna populations are reduced by application of granular nematicides/insecticides at planting (Scholte, 1987; Hofman, 1988) suggests that these organisms are present in soil at suppressive levels early in spring when soil temperatures are low.

These experiments showed that the mycophagous soil fauna is effective in reducing stem infection by *R. solani* at 10 °C as well as at 15 °C. This activity of the mycophagous soil fauna at low temperatures may provide a control method for *R. solani* if the populations of these organisms can be manipulated by cultural practices. The advantage of these mycophagous mesofauna is that they already form part of the soil ecosystem, they are adapted to the environmental conditions and there is no need to introduce them to the soil. Moreover, the endemic soil animals are not dependent solely on *R. solani* as a food source, so it is possible to achieve high densities before *R. solani* develops.

Chapter 4

Effect of soil moisture content on the suppression of Rhizoctonia stem canker on potato by the nematode *Aphelenchus avenae* and the springtail *Folsomia fimetaria*

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Effect of soil moisture content on the suppression of Rhizoctonia stem canker on potato by the nematode *Aphelenchus avenae* and the springtail *Folsomia fimetaria*

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Chapter 4

Effect of soil moisture content on the suppression of Rhizoctonia stem canker on potato by the nematode *Aphelenchus avenae* and the springtail *Folsomia fimetaria*

M. LOOTSMA and K. SCHOLTE

SUMMARY

The effect of soil moisture content on the suppression of Rhizoctonia stem canker on potato by mycophagous soil animals was studied in growth chambers. Three soil moisture levels were established in two bio-assays, in which potato sprouts grew through a 15 cm soil layer inoculated with sclerotia of *Rhizoctonia solani* (AG-3). In one experiment two levels of *R. solani* inoculum were applied. The effect on plant disease of mycophagous soil fauna was assessed by adding the springtail *Folsomia fimetaria* and/or the nematode *Aphelenchus avenae* to the soil.

In the absence of mycophagous organisms, Rhizoctonia disease severity on potato stems was highest in dry soil. *A. avenae* and *F. fimetaria* reduced Rhizoctonia stem canker when applied at populations found in the field. They were effective over a broad range of soil moistures. The stimulatory effect of dry soil conditions on Rhizoctonia stem canker was counteracted by a greater efficacy of the mycophagous soil fauna under these conditions. Mild drought stress did not seem to be a limiting factor in the biological control of stem canker by these two organisms.

INTRODUCTION

Mycophagous springtails and nematodes can suppress root rot fungi when tested in pots (Curl, 1979; Ulber, 1983; Barnes *et al.*, 1981). Hofman (1988) and Hofman *et al.* (1990) showed that

these mycophagous soil organisms can also reduce Rhizoctonia stem canker on potato under field conditions. However, less information is available about the effects of environmental conditions on the interaction between *R. solani* and the mycophagous soil fauna. This knowledge can be useful for optimizing the suppressive activity of the mycophagous soil fauna. The two experiments described in this paper focus on the effect of soil moisture. To investigate this a bio-assay was developed, using the springtail *Folsomia fimetaria* and the mycophagous nematode *Aphelenchus avenae* as test organisms for the *R. solani* suppressing soil fauna (Lootsma & Scholte, 1997a).

Hide & Firmager (1989) showed that *R. solani* is most virulent under dry conditions. Infection of potato sprouts was greater at a soil moisture content of 45% of the water holding capacity than at higher soil moisture contents. However, *A. avenae* appears inactive during dry periods, because the nematode coils and enters into anhydrobiosis (Demeure *et al.*, 1979). Low soil moisture can also cause migration and bioecological adaptations of edaphic collembola (Butcher *et al.*, 1971) but no information is available about their feeding activity under dry conditions. Therefore, in this study we investigated whether the suppressive activity of mycophagous soil fauna continues under dry soil conditions.

MATERIALS AND METHODS

Test procedure

Effects of mycophagous soil fauna on stem infection of potato by *R. solani* were studied using a special observation system (Lootsma & Scholte, 1997a). Twenty potato sprouts of cv. Element grew for 4-5 weeks through a 15 cm thick layer of a soil mixture of pure sand and pasteurized peat soil (the mixture had a pH-KCl of 5.5 and 5.0% organic matter; further details: Chapter 3), which had been inoculated two weeks earlier with *R. solani* and various populations of the springtail *F. fimetaria* and/or the nematode *A. avenae*. These animals were added together with a small amount of the medium in which they had been reared. The medium comprised potting soil with wheat flour, in which *Alternaria porri* was cultured as a food base for the mycophagous fauna. At the end of the experiment Rhizoctonia stem infection was scored.

Preparation of soils with three moisture levels

A soil mixture of 75 % pure sand and 25 % (v/v) enriched and pasteurized peat soil with a

moisture content of c. 10 % was used as the basic material. The soil mixture was divided into three samples. One sample was left at 10% moisture content (pF 2.9), another was wetted up to 14% moisture content (pF 2.0) and the third was air-dried to 7% moisture content (pF 3.8). Before filling the experimental containers, each soil sample was homogenized by mixing in a concrete mixer. During the experiments, the soil was frequently sampled to assess the water content, water was added to maintain the desired moisture content.

Experiment 1

One hundred sclerotia of *R. solani* were buried c. 10 cm deep in the soil, in a single line on the longitudinal axis in the middle of the container. This resulted in two groups of potato stems growing through the soil layer at different distances from the inoculum source. Within each container, two rows of five sprouts grew at 3 cm and two rows of five sprouts grew at 10 cm distance from the inoculum source. Thus both groups of stems were subjected to a different *R. solani* inoculum pressure. For each soil moisture level, soil fauna were added as follows: 1) 0 or 250 *F. fimetaria* per litre soil, 2) 0 or 7250 *A. avenae* per litre soil. All combinations of factor levels were applied, resulting in 3 moisture levels x 2 *F. fimetaria* levels x 2 *A. avenae* levels x 2 distances of *R. solani* inoculum = 24 treatments. Experimental units comprised ten potato sprouts. The experiment was carried out according to a split plot design with 6 replications, with soil moisture and soil fauna in the main plots. The six blocks were divided between two growth chambers, each at 12 ± 0.5 °C.

Experiment 2

In this experiment, sclerotia of *R. solani* were mixed uniformly through the soil at Day 1. The effect of *R. solani* inoculum density was tested by adding 15 or 30 sclerotia per litre soil. Moisture levels were the same as in Experiment 1. Mycophagous soil fauna were applied at: 1) 0 or 250 *F. fimetaria* per litre soil; 2) 0 or 20,000 *A. avenae* per litre soil. All combinations were applied, resulting in 2 x 3 x 2 x 2 = 24 treatments. Each experimental unit comprised one container with 20 potato sprouts. The experiment was carried out as a randomized block design with 4 replicates. The 4 blocks were divided between two growth chambers, each at 12 ± 0.5 °C.

Records

Stem infection by *R. solani* was measured two weeks after 50 % emergence and transformed to

a disease index (DI = 0 - 100) based on 10 stems (Exp. 1) or 20 stems (Exp. 2) (Lootsma & Scholte, 1997a). Populations of *F. fimetaria* and *A. avenae* were determined at planting (Day 14) and at the end of the experiments (Exp. 1: Day 53; Exp. 2: Day 42). Randomly selected soil samples of each experimental unit were mixed and 50 ml soil was used for determination of *A. avenae* and 2 samples of 55 ml for determination of *F. fimetaria*. Extraction and counting of *F. fimetaria* and *A. avenae* was done as described previously by Lootsma & Scholte (1997a).

Statistics

Populations of *F. fimetaria* and *A. avenae* and disease indices were analyzed using ANOVA. When necessary, square root transformations were applied to obtain normal distributions of observations.

RESULTS

Experiment 1

Potato sprouts 10 cm from the inoculum source showed little infection, with the disease-index varying from 0 to 0.4. Therefore, only data for treatments 3 cm from the inoculum source were examined (Table 1). The disease-index was higher under dry than under intermediate or wet soil conditions, when *A. avenae* was not introduced into the soil. Addition of *F. fimetaria* reduced disease severity at all soil moisture levels, whereas *A. avenae* only reduced disease severity under dry conditions.

Fourteen days after introduction, numbers of *F. fimetaria* were unchanged (Table 2). However, 53 days after introduction, *F. fimetaria* numbers were increased when in the presence of *A. avenae*, especially under wet conditions. Numbers decreased with increasing pF-value.

On Day 14, numbers of *A. avenae* had declined below the number introduced, and were significantly ($P<0.05$) lower at pF 2 than at pF 2.9; numbers at pF 3.8 were intermediate (Table 3). On Day 53, numbers of *A. avenae* were considerably higher than introduced, in all six treatments; the extent of the increase varied between treatments. At the lowest pF value, numbers of *A. avenae* were greatest in the absence of *F. fimetaria*.

Experiment 2

The four-factor interaction *R. solani* infestation * Soil moisture * *F. fimetaria* * *A. avenae* was not significant. However, three three-factor interactions were significant or tended to be

Table 1. Effect of *F. fimetaria* and *A. avenae* on Rhizoctonia disease index (0-100) of potato stems, at 3 cm from sclerotia, at three soil moisture levels (pF-values). Experiment 1

pF	- *F. fimetaria*		+ *F. fimetaria*	
	- *A. avenae*	+ *A. avenae*	- *A. avenae*	+ *A. avenae*
2.0	15	16	2	5
2.9	17	15	6	9
3.8	28	17	13	5
SED (55 d.f.)		4.2		

Main effect of *F. fimetaria* ($P < 0.001$) and the interaction Soil moisture * *A. avenae* ($P < 0.01$) are significant.

Table 2. Effect of soil moisture (pF value) on numbers of *F. fimetaria*/l soil, at 14 or 53 days after introduction of 250 springtails/l, in the presence or absence of *A. avenae*. Experiment 1

pF	Day 14		Day 53	
	- *A. avenae*	+ *A. avenae*	- *A. avenae*	+ *A. avenae*
2.0	235	235	369	1253
2.9	271	228	242	984
3.8	228	354	172	579
SED (25 d.f.)	74		227	

On Day 53 the main effects of Soil moisture ($P < 0.05$) and *A. avenae* ($P < 0.001$) are significant.

Table 3. Effect of soil moisture (pF value) on numbers of *A. avenae* ($\times 10^{-3}$)/l soil, at 14 or 53 days after introduction of 7.3×10^3 nematodes/l soil, in the presence or absence of *F. fimetaria*. Experiment 1

pF	Day 14		Day 53	
	- *F. fimetaria*	+ *F. fimetaria*	- *F. fimetaria*	+ *F. fimetaria*
2.0	1.6	2.2	42.1	22.5
2.9	3.5	3.9	33.8	35.3
3.8	3.1	2.8	38.1	28.0
SED (25 d.f.)	0.92		6.36	

On Day 14 main effect of Soil moisture is significant ($P < 0.05$) and at Day 53 the interaction Soil moisture * *F. fimetaria* is nearly significant at 5% ($P = 0.088$).

significant. These interactions are presented in Tables 4, 5 and 6.

In the absence of *F. fimetaria* and *A. avenae*, disease severity was generally lower under wet soil conditions (pF 2.0) than under drier soil conditions (pF 2.9 or 3.8) (Tables 4, 5 and 6).

Disease severity was approximately twice as high when soil was inoculated with 30 as with 15 sclerotia per litre soil, in the absence *F. fimetaria* (Table 4) or *A. avenae* (Table 5). When soil was infested with 15 sclerotia per litre, *F. fimetaria* reduced *R. solani* infection considerably at soil pF's 2.9 and 3.8, but less so at pF 2.0 (Table 4). However, when soil was infested with 30 sclerotia per litre, *F. fimetaria* was most effective at pF's 2.0 and 3.8 and least effective at pF 2.9. *A. avenae* reduced stem infection most at pF's 2.9 and 3.8, with 15 sclerotia per litre soil (Table 5). However, with 30 sclerotia per litre soil, *A. avenae* was only effective at pF 2.9. Effects of *Folsomia* springtails and *Aphelenchus* nematodes on the reduction of Rhizoctonia stem infection were additive at pF-values 2.0 and 2.9 (real effect vs expected effect were -35 vs -39 and -46 vs -46, respectively), but were significantly ($P<0.05$) antagonistic at pF 3.8 (-42 vs -65) (Table 6).

Fourteen days after introduction, numbers of *F. fimetaria* were lower than the numbers applied in all treatments; however, numbers were significantly higher in the presence than in the absence of *A. avenae* (Table 7). Soil moisture did not affect numbers. At the end of the experiment (Day 42), numbers of *F. fimetaria* were increased in the presence of *A. avenae*. On average, highest populations were found at the intermediate soil moisture level ($P<0.05$).

In contrast to *F. fimetaria*, at 14 days after introduction numbers of *A. avenae* had increased above the levels introduced and remained high until the end of the experiment (Table 8). On Day 14, numbers were highest at soil pF 3.8 and also at pF 2.9 if *F. fimetaria* was introduced ($P<0.01$). On Day 42, the positive effect of *F. fimetaria* also occurred at pF's 2.0 and 3.8 ($P<0.05$), but not at pF 2.9.

DISCUSSION

In both experiments, *R. solani* disease incidence was considerably higher under dry soil conditions than under wet soil conditions, in the absence of mycophagous soil fauna; this is in

Table 4. Effect of *F. fimetaria* on Rhizoctonia disease index (0-100) of potato stems, at three soil moisture levels (pF) and two levels of *R. solani* inoculation; averaged over - and + *A. avenae*. Experiment 2

pF	15 *R. solani* sclerotia/l soil		30 *R. solani* sclerotia/l soil	
	- *F. fimetaria*	+ *F. fimetaria*	- *F. fimetaria*	+ *F. fimetaria*
2.0	26	17	59	31
2.9	48	18	77	64
3.8	36	16	77	51
SED (69 d.f.)		5.2		

The three-factor interaction Soil moisture * *R. solani* * *F. fimetaria* is significant ($P<0.05$).

Table 5. Effect of *A. avenae* on Rhizoctonia disease index (0 - 100) of potato stems, at three soil moisture levels (pF) and two levels of *R. solani* inoculation; averaged over - and + *F. fimetaria*. Experiment 2

pF	15 *R. solani* sclerotia/l soil		30 *R. solani* sclerotia/l soil	
	- *A. avenae*	+ *A. avenae*	- *A. avenae*	+ *A. avenae*
2.0	29	14	53	53
2.9	44	22	84	57
3.8	40	12	70	60
SED (69 d.f.)		5.2		

The three-factor interaction Soil moisture * *R. solani* level * *A. avenae* is nearly significant at 5% ($P = 0.068$).

Table 6. Effect of *F. fimetaria* and *A. avenae* on Rhizoctonia disease index (0 - 100) of potato stems, at three soil moisture levels (pF); averaged over two *R. solani* inoculum levels. Experiment 2

pF	- *F. fimetaria*		+ *F. fimetaria*	
	- *A. avenae*	+ *A. avenae*	- *A. avenae*	+ *A. avenae*
2.0	52	34	31	17
2.9	75	50	54	29
3.8	72	41	38	30
SED (69 d.f.)		5.2		

The three-factor interaction Soil moisture * *F. fimetaria* * *A. avenae* is nearly significant at 5% ($P = 0.068$).

Table 7. Effect of soil moisture (pF) on numbers of *F. fimetaria*/l soil at 14 or 42 days after introduction of 250 springtails/l in the presence or absence of *A. avenae*. Experiment 2

pF	Day 14		Day 42	
	- *A. avenae*	+ *A. avenae*	- *A. avenae*	+ *A. avenae*
2.0	79	135	42	284
2.9	67	154	105	318
3.8	59	131	36	200

On Day 14 and Day 42 there is a significant effect of *A. avenae* ($P < 0.05$ and $P < 0.001$, respectively with square root transformed values). On Day 42 there is also a significant soil moisture effect ($P < 0.05$ with square root transformed values).

Table 8. Effect of soil moisture (pF) on numbers of *A. avenae* (x 10^{-3})/l soil at 14 or 42 days after introduction of 20 x 10^3 nematodes/l, in the presence or absence of *F. fimetaria*. Experiment 2

pF	Day 14		Day 42	
	- *F. fimetaria*	+ *F. fimetaria*	- *F. fimetaria*	+ *F. fimetaria*
2.0	27	23	22	33
2.9	24	38	27	22
3.8	46	40	19	29

On Day 14 there is a significant interaction between Soil moisture and *F. fimetaria* ($P < 0.05$ with square root transformed values) and on Day 42 the effect of *F. fimetaria* tends to significance ($P = 0.086$ with square root transformed values).

full agreement with findings of Hide & Firmager (1989).

In the two experiments, different methods were used to achieve differences in the inoculum pressure of *R. solani*. In Experiment 1, *R. solani* sclerotia were concentrated in one line, resulting in two groups of potato sprouts, differing in distance from the inoculum source. Sprouts 10 cm from the inoculum source were hardly infected, whereas those at 3 cm showed a moderate infection. This suggests that there was little mycelial outgrowth from sclerotia in the first 2-3 weeks of the experiment, possibly due to lack of nutrients. In this system, suppression

of *R. solani* by the mycophagous soil fauna may have taken place only after the fungus had colonized the plants.

In both experiments, *A. avenae* was most effective in suppressing potato stem infection under dry soil conditions. This effect could be explained as follows: 1) during the first weeks after introduction, populations of *A. avenae* increased slightly more under dry conditions than under wet conditions, 2) under dry conditions there will be a greater nutrient gradient between the non-rhizosphere soil and the rhizosphere, resulting from increased exudation and microbial activity in the rhizosphere of drought stressed potato plants (Curl & Truelove, 1986). This would encourage *A. avenae* to move to the root surface of the potato plants, where they can suppress *R. solani* more effectively. Hofman & Jongbloed (1988) found that the lesion on a potato stem was proportional to the size of an infection cushion. Hofman (1988) stated that mycophagous nematodes reduces the size of infection cushions by grazing. Under wet circumstances, it is more likely that the nematodes will continue feeding outside of the rhizosphere. In this bio-assay *A. avenae* was added to the soil together with the fungus *Alternaria porri* on which it was reared (Lootsma & Scholte, 1997a); this fungus could serve as an alternative food source.

The effect of soil moisture on the efficacy of *F. fimetaria* in reducing *R. solani* stem infection was less pronounced than with *A. avenae*. When *R. solani* infection levels were low (Experiment 1) or intermediate (Experiment 2: 15 sclerotia/l soil), *F. fimetaria* reduced stem infection more or less independently of soil moisture conditions. However, at high *R. solani* infection levels (Experiment 2: 30 sclerotia/l soil) *F. fimetaria* reduced disease severity more under wet conditions (pF 2.0) than under dry conditions (pF 2.9 and 3.8). *F. fimetaria* reproduced better under wet than under dry soil conditions. At the end of both experiments lowest numbers were found at pF 3.8. However, the correlation between numbers in the soil and suppressive effect was weak. Congregation of springtails on the root surface under dry soil conditions has been recorded by Wiggins & Curl (1979). It is therefore likely that a decreased efficacy by lower numbers partly is compensated by congregation of springtails at the root surface under dry soil conditions.

In Experiment 1 the combined application of *F. fimetaria* and *A. avenae* had no additional effect on *R. solani* infection under wet and normal conditions, because *F. fimetaria* alone had already reduced stem canker to a very low level. However, under dry conditions with a higher level of *R. solani*, there was an additive effect of *A. avenae*. In Experiment 2 with much higher infection levels by *R. solani*, joint application of *F. fimetaria* and *A. avenae* also had an additive

effect under wet and normal conditions, but an antagonistic effect under dry conditions. Under dry soil conditions *R. solani* grows very well, and when under such conditions soil infestation is high, mycophagous animals probably fail to overcome *R. solani* sufficiently.

In both experiments the population of *F. fimetaria* was increased in joint application with *A. avenae*. Similar effects were noticed by Bollen *et al.* (1990). The effect could be due to feeding of *F. fimetaria* on the fungus *A. porri*, that was introduced together with *A. avenae*. Direct feeding of *F. fimetaria* on *A. avenae* could also play a role. Edaphic springtails generally feed on decayed and undecayed plant material, fungi, and bacteria (Christiansen, 1964) but predation of nematodes has also been recorded (Gilmore & Potter, 1993). However, numbers of *A. avenae* were not reduced at pF 3.8.

In summary *A. avenae* and *F. fimetaria* were both capable of reducing Rhizoctonia stem canker when applied at populations that occur in the field. They were effective over a broad range of soil moistures. The stimulatory effect of dry soil conditions on Rhizoctonia stem canker were counteracted by a greater efficacy of the mycophagous soil fauna in reducing stem infection under these conditions. This indicates that mild drought stress is not a limiting factor in the biological control of Rhizoctonia stem canker by mycophagous soil fauna.

Chapter 5

Effect of soil pH and amendments with dried fodder rape on mycophagous soil animals and Rhizoctonia stem canker of potato

Submitted as:

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Effect of soil pH and amendments with dried fodder rape on mycophagous soil animals and Rhizoctonia stem canker of potato

Chapter 5

Effect of soil pH and amendments with dried fodder rape on mycophagous soil animals and Rhizoctonia stem canker of potato

M. LOOTSMA and K. SCHOLTE

SUMMARY

Effects of adding dried rape material to the soil and of soil pH on the ability of mycophagous springtails and nematodes to suppress stem canker on potato, caused by *Rhizoctonia solani*, were investigated in growth chambers. A bio-assay was used with the springtail *Folsomia fimetaria* and the nematode *Aphelenchus avenae* as test organisms.

Rape material, its age and the soil pH affected the suppressive ability of *F. fimetaria* and *A. avenae* significantly. Numbers of *F. fimetaria* and *A. avenae* were increased when plant material was incorporated into the soil. *F. fimetaria* reduced stem canker under a broad range of conditions, but when rape was added to the soil at pH-KCl 6.2 its suppressive effect disappeared completely, probably because of the presence of alternative food sources. *A. avenae* only reduced stem canker at relatively high populations.

INTRODUCTION

Rhizoctonia solani-AG3 is a serious pathogen of potato, causing stem canker and stolon pruning. Infections can occur from both tuber- and soil-borne inoculum. Soil-borne inoculum can cause severe plant infections resulting in lower tuber yields and tuber quality (Scholte, 1989), especially in short rotations (Scholte, 1987).

R. solani survives in the soil by sclerotia. Plant infections can be reduced by fungal and bacterial antagonists (Chand & Logan, 1984; Jager & Velvis, 1985). However, these antagonists

failed more or less to control stem and stolon canker when applied in field experiments. Plant infections can also be suppressed by the mycophagous soil mesofauna (Hofman, 1988; Bollen *et al.*, 1990). Stimulation of these suppressive animals in the soil could be used as a biological control method. These mycophagous animals have a broad range of food sources: not only *R. solani* is used, but also other fungi, providing the possibility to increase the population densities of mycophagous soil animals by an increased biological soil activity.

Edwards & Lofty (1969), Van de Bund (1970) and Sievers & Ulber (1990) found that large amounts of decaying organic material in the soil stimulate the biological soil activity, including the mycophagous soil fauna. At low pH, especially saprophytic fungi contribute to the decomposition of organic material, whereas with increasing pH the bacterial activity increases (Bååth *et al.*, 1980; Feest & Campbell, 1986). This change in dominance of saprophytic organisms could affect the number of mycophagous soil animals and their effect on *R. solani*, because these saprophytes also serve as a food supply for the soil animals.

The composition of the organic material may also affect biological soil composition (Chung *et al.*, 1988). Young organic material with a low C/N ratio and a low cellulose content will decompose much faster than old material (Vilsmeier & Gutser, 1988). However, the saprophytic activity of *R. solani* may also be stimulated by organic material, resulting in higher disease ratings (Baker & Martinson, 1970; Wall, 1984; Moubasher & Abdel Hafez, 1980). Chung *et al.* (1988) found that especially organic material with high cellulose contents enhanced *R. solani*.

To elucidate the effect of soil amendments with organic materials and the effect of soil pH on the population of mycophagous soil fauna and on the incidence of Rhizoctonia plant infections, three experiments were conducted in growth chambers. In these experiments a bio-assay was used, previously described by Lootsma & Scholte (1997a). As test organisms for the mycophagous soil fauna, the springtail *Folsomia fimetaria* and the nematode *Aphelenchus avenae* were used, because they are important representatives of the mycophagous fauna in agricultural soils (Hofman *et al.*, 1990). Dried material of rape (*Brassica napus* spp. *oleifera*) was chosen as organic amendment, because rape is commonly used as a green manure crop in Dutch agriculture.

MATERIALS AND METHODS

Test procedure

Effects of mycophagous soil fauna on Rhizoctonia stem canker of potato were studied using a special observation system (Lootsma & Scholte, 1997a). Twenty potato sprouts of cv. Element were grown for 4 - 5 weeks through a 15 cm thick layer of a mixture of 75% pure sand and 25% (v/v) enriched (nutrients + clay) and pasteurized peat soil (the mixture had a pH-KCl of 5.5 and 5.0% organic matter; further details: Chapter 3), which had been inoculated two weeks earlier with 30 sclerotia/l soil of *R. solani* and various populations of the springtail *F. fimetaria* and/or the nematode *A. avenae*. The experiments were carried out in growth chambers at 12 °C. During the experiment soil moisture was maintained at pF 2.7 - 3.1 by covering the containers with a plastic foil before emergence of the sprouts and by daily wetting after emergence. At the end of the experiment, Rhizoctonia stem infections were scored.

Preparation of *R. solani* inoculum, rearing of *F. fimetaria* and *A. avenae*, methods of inoculation and determination of Rhizoctonia stem infection have been described in detail by Lootsma & Scholte (1997a).

Experiment 1

Two different types of rape material were mixed through the soil: young or old material. The young material consisted of leaves and stems of six-weeks-old rape plants, which had been fertilized with large amounts of nitrogen. The old material consisted of the leaves and stems of seven-months-old plants, that had been sown in August and were harvested in March. These plants had not received nitrogen fertilizer. The material was dried at 105 °C and then cut into pieces with a maximum length of 5 cm.

On Day 1 the soil was inoculated with 30 sclerotia of *R. solani* per litre. The following factors were varied; 1) rape addition: 0, 1.33 g/l young material or 1.33 g/l old material, 2) *F. fimetaria*: 0 or 400 springtails/l soil, 3) *A. avenae*: 0 or 40,000 nematodes/l soil. Sclerotia of *R. solani* and the rape material (applied two weeks before Day 1) were thoroughly mixed through to the soil using a concrete mixer. All combinations of factor levels were applied, resulting in a factorial experiment with 3 x 2 x 2 = 12 treatments. The experiment was carried out as a randomized block design with 4 replicates.

Experiments 2 and 3

In Experiments 2 and 3, two different soil pH-KCl levels of 4.8 and 6.2 were established in the soil. The mixture of peat soil and pure sand originally had a pH-KCl of 5.5. A pH-KCl of 4.8 was obtained by thoroughly mixing a 0.5 M-H_2SO_4 solution through the soil, and a pH-KCl of 6.2 was obtained by thoroughly mixing of $Ca(OH)_2$ through the soil. The relation between the addition of the chemicals and the final pH was assessed in preliminary experiments. At the same date of establishing the pH levels, two levels of organic rape materials were applied to the soil: no rape or 1.33 g of a mixture of young and old rape material (see Experiment 1). Three weeks after mixing the rape material through the soil, the soil was inoculated with *F. fimetaria*, *A. avenae* and *R. solani* (30 sclerotia/l). The date of inoculation was considered as Day 1 in the experiment. Thus, the following factors were varied; 1) pH-KCl: 4.8 or 6.2, 2) rape material: 0 or 1.33 g/l, 3) *F. fimetaria*: 0 or 250 springtails/l soil, 4) *A. avenae*: 0 or 14,000 nematodes/l soil. This resulted in a factorial with 2 x 2 x 2 x 2 = 16 treatments. The experiment was carried out as a randomized block design with four replicates. The pH-KCl levels 4.8 and 6.2 were checked 3 weeks after applying H_2SO_4 or $Ca(OH)_2$ (they were still 4.8 and 6.2, respectively) and at the end of the experiment (respectively they were 5.1 and 5.9, in Experiment 2, and 4.7 and 6.3, respectively, in Experiment 3). Furthermore, Experiment 3 was a complete replicate of Experiment 2.

Records

The severity of stem infection caused by *R. solani* on the subterranean part of each potato stem was assessed on Day 42 (Experiment 1) or Day 49 (Experiments 2 and 3). Disease severities of all stems were transformed to a disease index (0-100) for each experimental unit with: 0 = no infection and 100 = all 20 stems completely infected (Lootsma & Scholte, 1997a).

On Day 14 and 42 (Experiment 1) or Day 21 and 49 (Experiments 2 and 3) populations of *F. fimetaria* and *A. avenae* were assessed. Sampling, extraction and counting was done as previously described by Lootsma & Scholte (1997a).

Statistical analysis

Rhizoctonia disease indices and populations of *F. fimetaria* and *A. avenae* were analyzed using ANOVA. As Experiment 3 was a complete replicate of Experiment 2, both experiments were analyzed together, treated as separate blocks in ANOVA. LSD-tests were performed for comparing means.

RESULTS

Experiment 1

In the absence of mycophagous soil fauna (control treatments), young rape material had no effect on the disease index, whereas old rape material stimulated Rhizoctonia stem infection significantly ($P<0.05$; Fig. 1). When added alone, *F. fimetaria* and *A. avenae* reduced stem infection significantly ($P<0.001$). However, for *F. fimetaria* this reduction was highest when no rape material was added, whereas *A. avenae* performed best when old rape material was applied

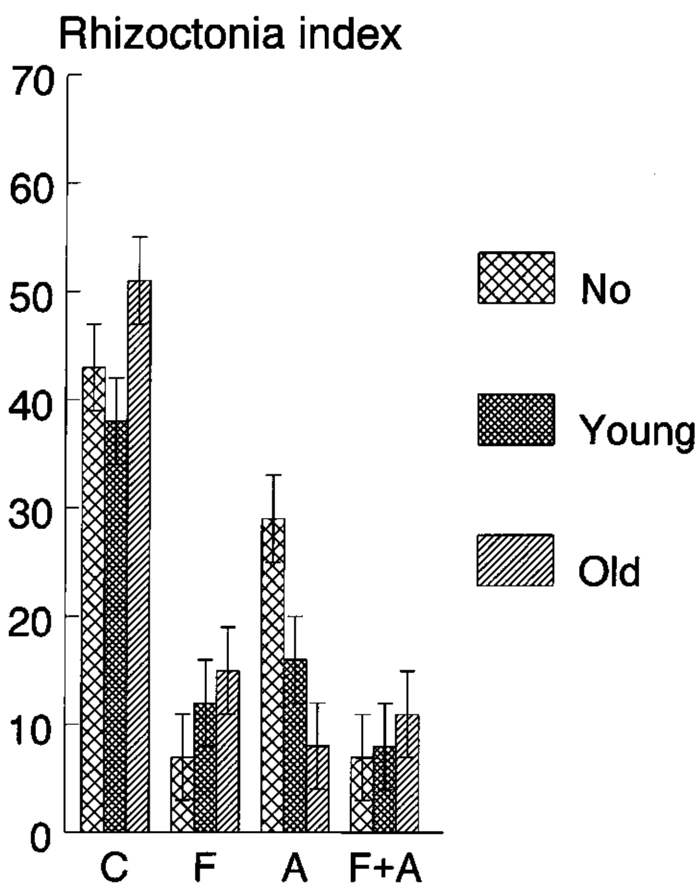

Fig. 1. Rhizoctonia disease index (0-100) of potato stems without (no) or with addition of young or old rape material in control (C) treatments and in treatments with *F. fimetaria* (F) and/or *A. avenae* (A). Experiment 1. Vertical bars indicate the LSD-values.

to the soil and poorest when no rape was added. The combination of *F. fimetaria* and *A. avenae* resulted in disease indices that were not significantly different from the treatments in which *F. fimetaria* was added alone.

Fourteen days after introduction, numbers of *F. fimetaria* had increased significantly ($P<0.001$) from Day 1, but treatments did not differ (Fig. 2). However, at the end of the experiment (Day 42) addition of rape resulted in higher numbers of *F. fimetaria*. Old rape material stimulated *F. fimetaria* more than young material ($P<0.05$).

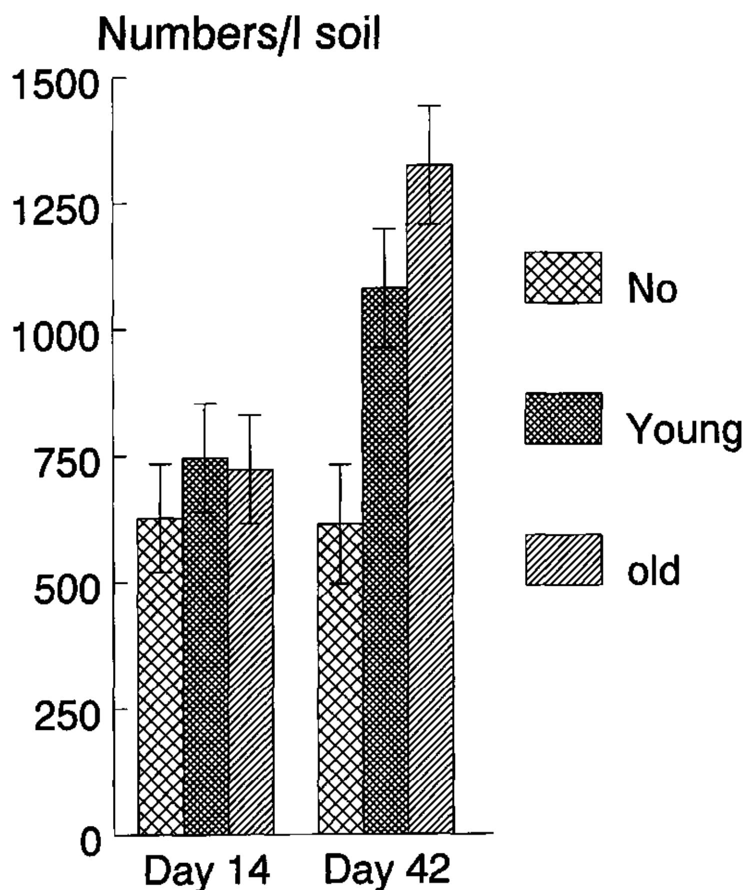

Fig. 2. Numbers of *F. fimetaria*/l soil after introduction of 400 springtails/l in treatments with no, young or old rape material in the soil. Experiment 1. Vertical bars indicate the LSD-values.

On Day 14, numbers of *A. avenae* were higher than introduced (Fig. 3). On Day 42, numbers of *A. avenae* were higher when rape material had been added. Old rape material stimulated *A. avenae* more than young material ($P<0.05$). From Day 14 to Day 42, numbers of *A. avenae* decreased slightly when no rape of young rape material was applied to the soil, but increased when old rape material was added.

Experiments 2 and 3

The mean disease index in Experiment 3 was higher than in Experiment 2 (Experiment 2: 30.7; Experiment 3: 71.7). However, the effects of the treatments on the disease severities and on the

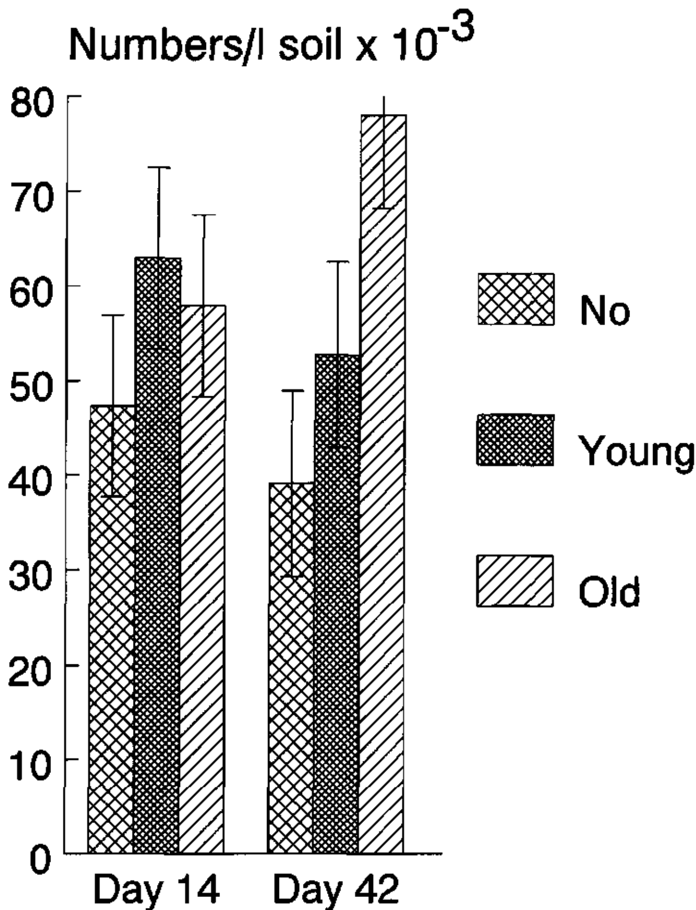

Fig. 3. Numbers of *A. avenae*/l soil after introduction of 40,000 nematodes/l in treatments with no, young or old rape material in the soil. Experiment 1. Vertical bars indicate the LSD-values.

population densities of the fungivorous organisms were very similar for these experiments: there were no statistically significant interactions between experiment and treatments.

At pH 4.8, addition of rape resulted in a higher disease index ($P<0.001$; Fig. 4). At this pH, *F. fimetaria* reduced the disease severity; however, the reduction was most pronounced when no rape was applied to the soil. At pH 6.2, *F. fimetaria* reduced stem infection ($P<0.001$) when no rape was applied, but this effect was absent with rape addition. *A. avenae* did not reduce the disease severity at all (data not shown in figures, the disease indices were 53 for untreated and 50 for *A. avenae*).

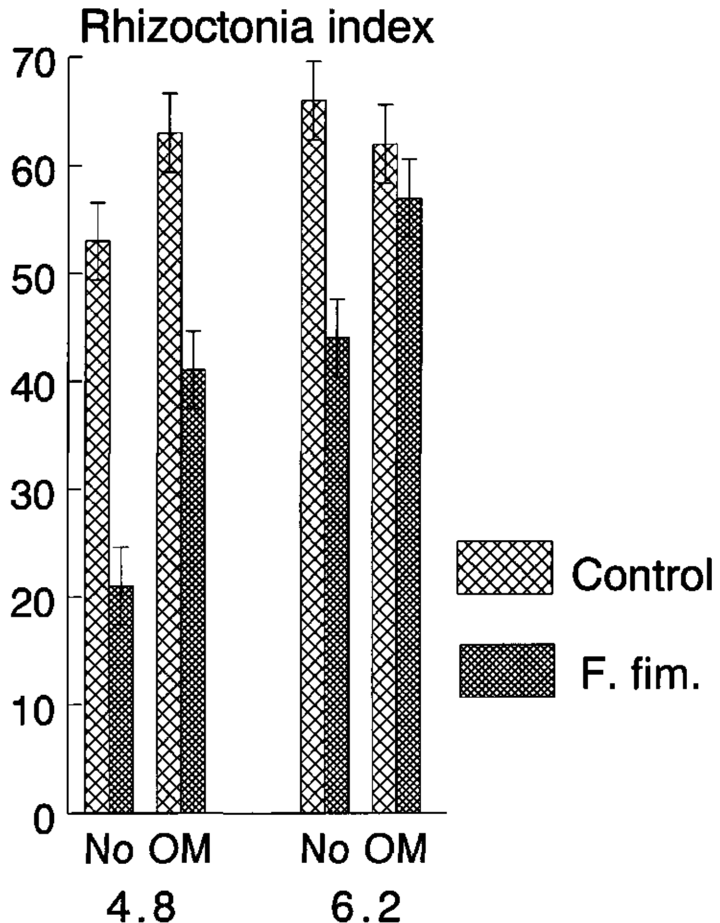

Fig. 4. Rhizoctonia disease index (0-100) of potato stems at two soil pH levels (4.8 or 6.2) without (No) or with addition of organic rape material (OM) to the soil in control treatments and treatments with *F. fimetaria*. Experiments 2 and 3. Vertical bars indicate the LSD-values.

On Day 49, numbers of *F. fimetaria* had decreased in all treatments compared with Day 21 (Fig. 5). Addition of rape had a positive effect on numbers of *F. fimetaria* at pH 4.8 ($P<0.05$), but a negative effect at pH 6.2.

Twenty-one days after introduction, numbers of *A. avenae* had decreased considerably in all treatments compared with the numbers inoculated (Fig. 6). However, numbers remained higher when organic material was applied to the soil, except at Day 21 at pH 6.2.

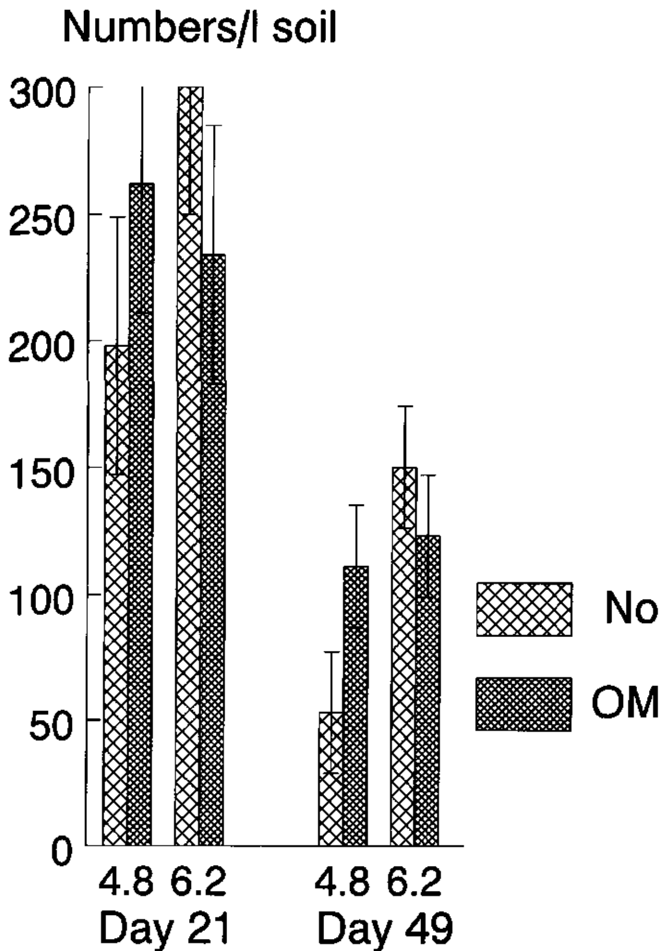

Fig. 5. Numbers of *F. fimetaria*/l soil 21 or 49 days after introduction of 250 springtails/l at two soil pH levels (4.8 or 6.2) in treatments without (No) or with organic rape material (OM) in the soil. Experiments 2 and 3. Vertical bars indicate the LSD-values.

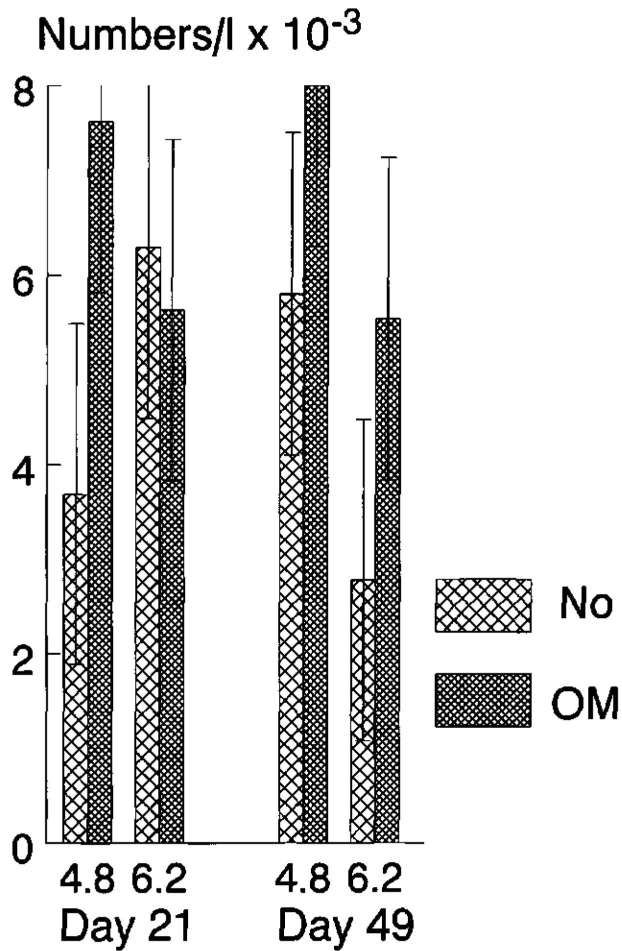

Fig. 6. Numbers of *A. avenae*/l soil 21 or 49 days after introduction of 14,000 nematodes/l at two soil pH levels (4.8 or 6.2) in treatments without (No) or with organic rape material (OM) in the soil. Experiments 2 and 3. Vertical bars indicate the LSD-values.

DISCUSSION

In contrast to young material, old rape material increased Rhizoctonia stem canker in the absence of mycophagous soil mesofauna. Old rape material contains a higher concentration of cellulose. Chung *et al.* (1988) found a stimulating effect of cellulose on the saprophytic activity

of *R. solani*.

Numbers of *F. fimetaria* were positively affected or remained higher in treatments with rape material in the soil compared with untreated soil, except in Experiment 2 at pH 6.2. In Experiment 1, the ability of *F. fimetaria* to reduce stem canker was the same (old rape) or even less (young rape) when rape was added, although numbers of *F. fimetaria* were higher in the treatments with rape. This means that the suppressive capacity per animal decreased. Although in Experiment 2 numbers of *F. fimetaria* slightly decreased after introduction, they maintained their capacity to reduce stem canker. The efficacy of *F. fimetaria* to reduce stem canker was highest at pH 4.8 without addition of rape material, though *Folsomia* numbers were higher when rape material was applied. However, soil amendment with rape at pH 6.2 resulted in a complete loss of their effectiveness. This was not caused by reduced numbers of *F. fimetaria*. Feeding preference of *F. fimetaria* for microbes and other fungi, probably abundantly present under these conditions, may cause this absence of *R. solani* suppression. Andrén & Schnuerer (1985) found that *F. fimetaria* fed for a great part on bacteria in the presence of decomposing barley straw. Christiansen (1964) reported that edaphic collembola generally feed on decayed and undecayed plant material, fungi and bacteria. Hagvar (1990) also pointed to the importance of food competition for understanding the effect of pH changes on soil fauna communities. Apparently, the efficacy of *F. fimetaria* to control *R. solani* seems to decrease when alternative food sources are available.

The ability of *A. avenae* to reduce stem canker in Experiment 1 was higher when rape was added to the soil. This was probably caused by the positive effect of rape on the population of *A. avenae*. In Experiments 2 and 3, numbers of *A. avenae* were also higher when rape was added to the soil compared with untreated soil. However, compared with Experiment 1, inoculated numbers of soil animals were lower in Experiments 2 and 3. Moreover, there was also a severe decline in numbers of *A. avenae* after introduction. Apparently, the population of *A. avenae* was too small for a significant reduction of stem canker in Experiments 2 and 3.

Numbers of mycophagous soil animals can be increased by the addition of organic material to the soil as was found in Experiment 1 and, especially at pH 4.8, in Experiments 2 and 3. The chemical and/or physical composition of the organic material also seems relevant. In Experiment 1, numbers of *F. fimetaria* and *A. avenae* increased more when old plant material was mixed with the soil than when young material was used. Though numbers of mycophagous soil animals can be increased by organic soil amendments, their efficacy in controlling *R. solani* probably also depends on the availability of alternative food sources. *F. fimetaria* has a greater preference for alternative food than *A. avenae*.

Chapter 6

Effect of farmyard manure and green manure crops on populations of mycophagous soil fauna and Rhizoctonia stem canker of potato

Submitted as:

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Chapter 6

Effect of farmyard manure and green manure crops on populations of mycophagous soil fauna and Rhizoctonia stem canker of potato

M. LOOTSMA and K. SCHOLTE

SUMMARY

Effects of organic soil amendments on populations of mycophagous springtails and nematodes and on *Rhizoctonia solani* stem canker of potato were investigated in two field experiments each lasting two years. The organic amendments consisted of three green manure crops (white mustard, forage rape and oats), and farmyard manure (FYM, alone or in combination with white mustard). In Year 1, before the application of soil amendments, experimental fields were infested with *R. solani* by growing a potato crop from seed tubers severely infested with black scurf. In the next year potato was grown again as test crop.

In Experiment 1, there was a moderate degree of Rhizoctonia stem infection in the test crop. Organic amendments reduced the disease severity and increased populations of mycophagous soil organisms. The greatest reduction in disease severity was found when FYM application was combined with white mustard or when oats was grown as green manure crop. In Experiment 2, Rhizoctonia stem infection was so severe that emergence of potatoes in the test crop was reduced. Again, soil fauna populations were increased by farmyard manure combined with mustard and also when oats was grown as green manure crop. The disease severity was only slightly reduced by the former treatment, and significantly by the latter one. FYM+white mustard increased the springtail populations and had no effect on mycophagous nematodes, whereas oats increased the numbers of mycophagous nematodes tenfold. The results from both experiments support the hypothesis that stimulating the populations of mycophagous soil mesofauna can contribute to a reduction in Rhizoctonia disease severity in potato.

INTRODUCTION

Application of granular insecticides/nematicides increases Rhizoctonia stem canker in various types of soil (Scholte, 1987; Hofman, 1988). Hofman *et al.* (1990) found that the increased level of stem canker on potato after soil disinfection with granular nematicides was caused by decreased populations of mycophagous springtails, mites and nematodes. This suggests that soil fauna may be present which are able to suppress *R. solani*. Stimulation of such organisms could be a strategy for bioecological control of Rhizoctonia canker.

Research on effects of various soil amendments including green manure crops on *R. solani* has mainly been focused on bacterial or fungal antagonists of the pathogen (Boosalis, 1956; Davey & Papavizas, 1963; Hoitink & Fahy, 1986; Naumann & Lange-de la Camp, 1977). However, organic amendments also stimulate the soil fauna (Edwards & Lofty, 1969; Sievers & Ulber, 1990; Watanabe & Ogawa, 1990). When the soil fauna suppressive to *R. solani* can also be stimulated by organic soil amendments they could be used to manipulate the populations of these animals.

This paper describes two field experiments, each lasting two years, designed to investigate the effects of various organic amendments on populations of mycophagous springtails and nematodes, and on Rhizoctonia stem canker of potato. The first year of an experiment was used to contaminate the soil with *R. solani* by growing a potato crop from seed tubers severely infested with black scurf. After the harvest of this potato crop in August, several treatments of organic matter supply were applied. In the next year potato was grown again as test crop.

Effects of soil amendments on populations of mycophagous mesofauna may depend on the characteristics of the amendment. Therefore, amendments were used varying in rate of decomposition (farmyard manure vs green manure crops), whilst green manure crops with different characteristics were used (white mustard: does not survive winter; oats: does not survive winter but has a very fine, extensive root system; rape: persists during winter). To estimate the suppressive effect of the mycophagous animals present in the soil, a treatment was included with the biocide aldicarb applied prior to planting potato as the test crop.

MATERIALS AND METHODS

Experimental set up

A field experiment lasting two years was carried out in 1991 - 1992 (Experiment 1) and repeated in 1993-1994 (Experiment 2). The experiments were located in Achterberg (near Wageningen) on a sandy soil (fractions: 3.2% <2 μm, 4.7% 2 - 50 μm and 92.2% 16 - 2000 μm) with a pH-KCl of 5.2 and an organic matter content of 3.9%. In Experiment 1, the preceding crops were potato (c. Prominent) in 1989 and sugar beet (c. Univers) in 1990 and in Experiment 2 potato (c. Prominent) in 1991 and maize (c. Brutus) in 1992. In both experiments potato was grown in two consecutive years.

In April of Year 1, pre-sprouted seed tubers of cv. Santé (Experiment 1) or Spunta (Experiment 2), obtained from a commercial seed production farm and severely infested with black scurf, were mechanically planted 30 cm apart at a row distance of 75 cm. Plots were 6 x 6 m^2, containing 8 rows of potato. In the first week of August the potato haulm was killed by diquat (Reglone, 200 g/l a.i., 5 l/ha) and tubers were harvested 2 - 3 weeks later.

From August 20 of Year 1 to April of Year 2 various treatments were applied to manipulate the mycophagous soil fauna (Table 1). Besides the real control treatment (no organic amendments or soil disinfectants) an additional control treatment was applied. In this extra control treatment soil was treated with the insecticide/nematicide aldicarb (Temik-10G, 10 % a.i., 30 kg/ha) broadcast and incorporated with a spring-tine cultivator one day before planting potato in Year 2 to reduce populations of mycophagous soil animals. Other treatments were applied to enhance populations of mycophagous mesofauna. In treatments with farmyard manure (FYM) application, 35 tonnes per hectare of one-year-old FYM was incorporated into the soil with a spading machine on August 20 of Year 1. The green manure crops white mustard (*Sinapis alba*, cv. Salvo, 16 kg/ha), forage rape (*Brassica napus* ssp. *oleifera*, cv. Ramon, 12 kg/ha) and oats (*Avena sativa*, cv. Adamo, 170 kg/ha) were sown in the last week of August in rows 12.5 cm apart. The debris of these crops was incorporated into the soil with a spading machine on March 16 of Year 2.

In Year 2 of the experiments potato was grown as test crop. The seed tubers (size 35 - 40 mm) of cv. Element were visibly free of black scurf and had been disinfected against *R. solani* by immersion for 10 seconds in a solution of validamycine (Solacol, AAgrunol, 30 g/l a.i., 3% solution of the trade product). On April 17 (Exp. 1) or April 15 (Exp. 2) seed tubers were planted by hand at a spacing of 20 x 75 cm to achieve a high stem density. Both experiments were carried out in a randomized complete block design with six replicates.

N, P, K and Mg fertilizers were applied each year at recommended quantities. Ridges

Table 1. Treatments in Experiments 1 and 2.

Treatment number	Treatment description	Green manure crop [a]	Farmyard manure incorporation[b]
1	Control	None	None
2	Aldicarb[c]	None	None
3	FYM	None	Yes
4	Mustard	Mustard	None
5	Rape	Rape	None
6	Oats	Oats	None
7	FYM+mustard	Mustard	Yes

[a] Green manure crops were sown on August 26/Year 1.

[b] Farmyard manure was incorporated on August 20/Year 1.

[c] Soil was treated with aldicarb one day before planting potato in April/Year 2.

were prepared directly after planting in both experiments. Weeds and late blight were controlled chemically. When necessary, fields were irrigated.

Records in Year 2

In Experiment 1 on April 16, five soil samples of 70 ml each were taken at a depth from 5 - 7.5 cm in undisturbed soil, using an auger 6 cm wide and 2.5 cm deep, to assess the population of springtails. On June 15, six plants of each plot were gently lifted and a mixed sample of roots + adhering soil (Hofman & s'Jacob, 1989) was collected. Of each sample 15 g roots + adhering soil was used to assess the population of mycophagous and saprophagous nematodes.

In Experiment 2 five plants of each plot were gently lifted on May 18. From each plant a sample of 70 ml of the root adhering soil was collected to assess the population of springtails. The nematode population was determined in a 15 g mixed sample of roots + adhering soil from the five potato plants. On June 15, samples were taken in a similar way in the control and aldicarb-treated plots (Treatments 1 and 2) to assess populations of mycophagous springtails and nematodes.

Extraction and counting the springtails and nematodes were done as described previously

by Lootsma & Scholte (1997a). Springtails were identified using a dissecting microscope at a magnification of 60x. Identification of nematodes took place on approximately 250 nematodes under a dissection microscope at a magnification of 200 - 600x. In contrast to Experiment 2, in Experiment 1 the saprophagous and mycophagous nematodes were not counted in separate groups.

On June 15, Rhizoctonia stem canker was assessed on 40 plants of the inner four rows of each plot and recorded as described by Lootsma & Scholte (1996).

Statistical analysis

Rhizoctonia disease ratings and populations of springtails and nematodes were analyzed using ANOVA. When necessary, log transformations were applied to obtain normal distributions. LSD-tests were performed for comparing means.

RESULTS

Springtails

In both experiments *Tullbergia krausbaueri* and *Folsomia fimetaria* were the predominant mycophagous springtails, averaged over all treatments 54 and 42 % of total number of springtails in Experiment 1 and 51 and 38 % in Experiment 2, respectively. The ratio between the two species was not significantly affected by the treatments, except when indicated in the text.

Table 2. Effect of aldicarb on relative numbers of mycophagous soil animals. Experiment 2

Treatment		May 18	June 15
1 Control		100	100
3 Aldicarb	*T. krausbaueri*	51	74
	F. fimetaria	14	22
	A. avenae + *Aphelenchoides* spp.	71	3

In Experiment 1, prior to planting potato, populations of mycophagous springtails had increased significantly (P<0.05) in plots where FYM had been incorporated (Fig. 1), but the increase was more pronounced when soil was amended with FYM + mustard as green manure crop. Especially populations of *F. fimetaria* were stimulated by FYM, from 32% of the total population in the control to 54% in the FYM treatments. Of the green manure crops only mustard increased populations of springtails (P<0.05). Effects of aldicarb on the population of springtails were not assessed in this experiment, because soil sampling preceded the application of the chemical.

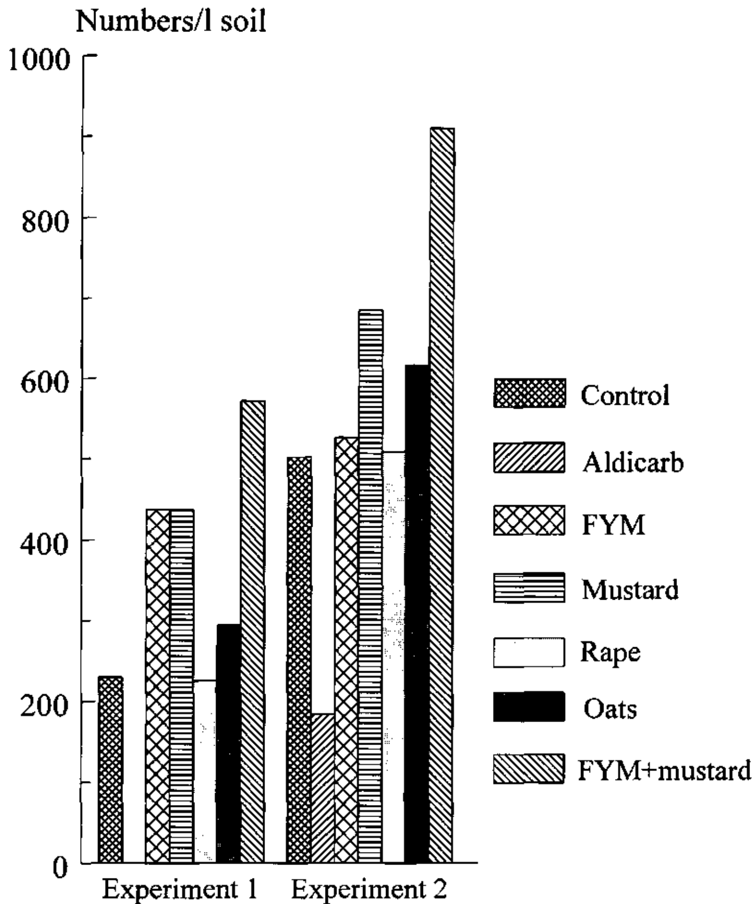

Fig. 1. Effect of soil treatments on populations of springtails (numbers/l soil) prior to planting of potato (Experiment 1) or 5 weeks after planting potato (Experiment 2) in Year 2. Significant effects after log transformations are indicated in the text.

In Experiment 2, springtail populations increased significantly ($P<0.05$) when FYM was applied in August combined with mustard as green manure crop (Fig. 1). Total number of mycophagous springtails was not affected by oats, but the population of *F. fimetaria* doubled significantly ($P<0.05$), whereas that of *T. krausbaueri* decreased. The population of springtails was reduced by aldicarb ($P<0.05$). *F. fimetaria* seemed more sensitive to aldicarb than *T. krausbaueri* (Table 2).

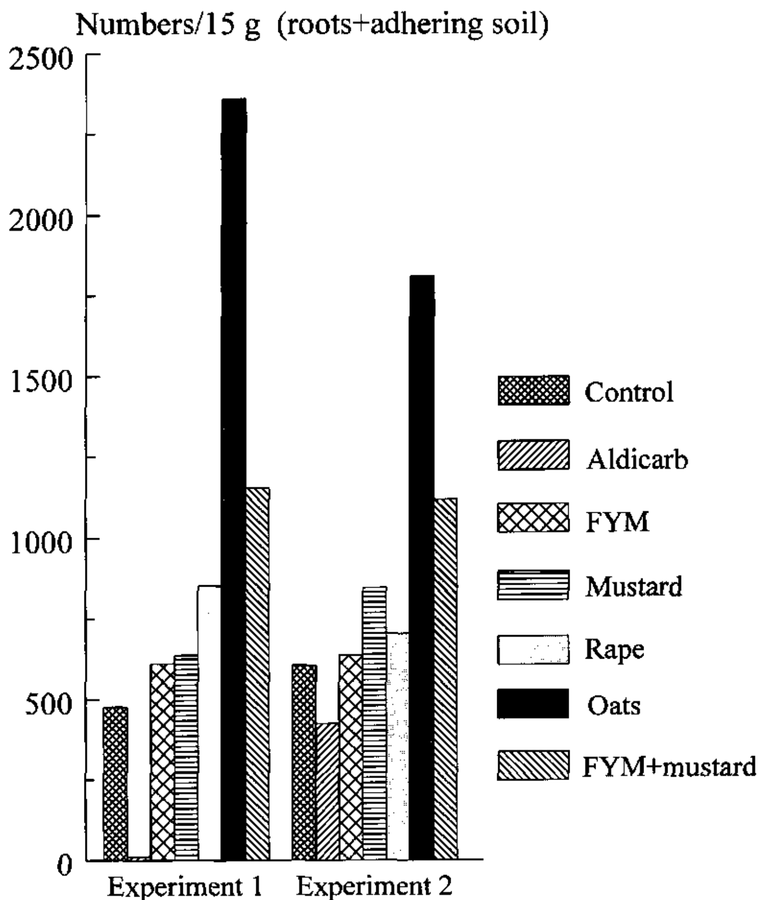

Fig. 2. Effect of soil treatments on populations of saprophagous + mycophagous nematodes (numbers/15 g roots + adhering soil) 8 weeks (Experiment 1) or 5 weeks (Experiment 2) after planting potato in Year 2. Significant effects after log transformations are indicated in the text.

Nematodes

In Experiment 1, the entire population of saprophagous + mycophagous nematodes was almost completely killed by aldicarb (Fig. 2). When FYM was incorporated into the soil combined with mustard as green manure crop, nematode populations increased significantly ($P < 0.05$). FYM application alone and mustard and rape as green manure crops did not significantly increase nematode populations. However, oats as green manure crop greatly increased numbers of nematodes ($P < 0.001$).

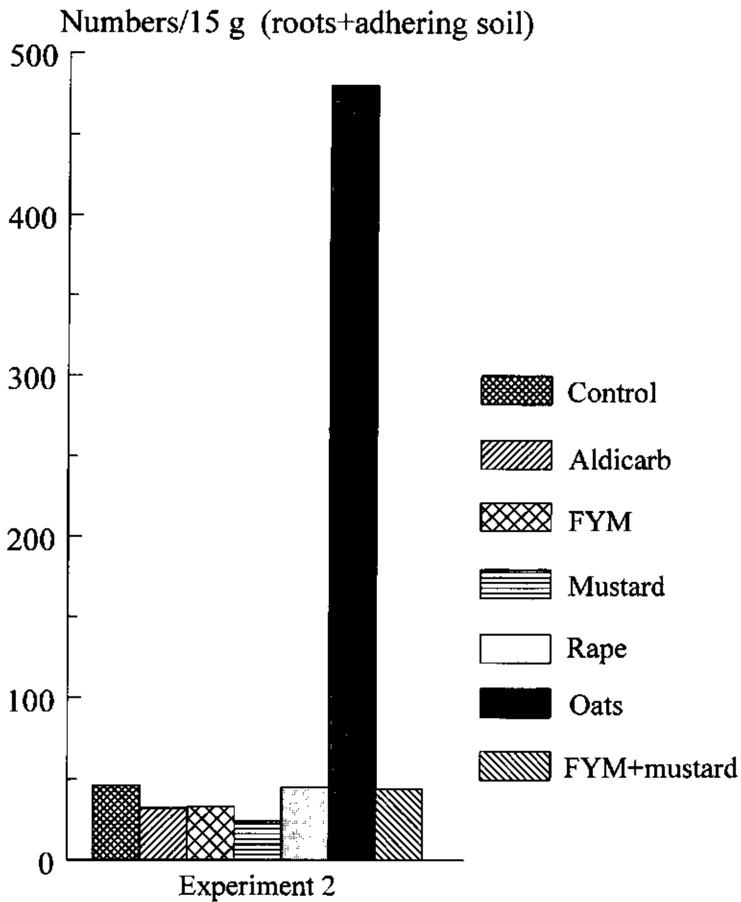

Fig. 3. Effect of soil treatments on populations of mycophagous nematodes 5 weeks after planting potato in Year 2. Experiment 2. Significant effects after log transformations are indicated in the text.

In Experiment 2, total number of mycophagous + saprophagous nematodes increased significantly ($P<0.05$) when soil was amended with FYM + mustard as green manure crop, and especially when oats was grown as green manure crop ($P<0.01$). However, the population of mycophagous nematodes only increased (and drastically so) under oats ($P<0.001$; Fig. 3). *Aphelenchus avenae* and *Aphelenchoides* spp. were by far the prevailing mycophagous species. Numbers of mycophagous nematodes were adversely affected by aldicarb, not significantly on May 18 (Table 2) but very appreciably on June 15.

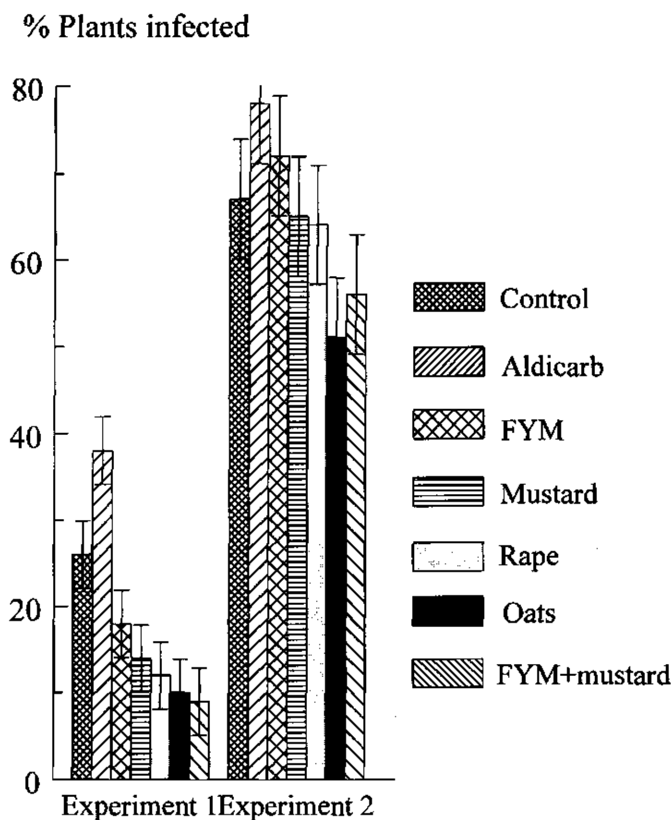

Fig. 4. Effect of soil treatments on percentage (%) potato plants moderately or severely infected by *R. solani*. Vertical bars indicate the LSD-values.

Rhizoctonia stem infection

In Experiment 1, soil disinfection with aldicarb stimulated significantly ($P<0.05$) Rhizoctonia canker (Fig. 4). Farmyard manure (FYM) and the green manure crops significantly ($P<0.05$) reduced the disease severity with the lowest value observed for oats. When FYM was combined with mustard as a green manure crop disease level was comparable to that of oats.

In Experiment 2, disease severity was much higher than in Experiment 1. Only oats reduced stem infection significantly ($P<0.05$).

DISCUSSION

The objective of these experiments was to find out whether populations of mycophagous soil animals could be stimulated by soil amendments with the aim to obtain a greater suppressiveness of the soil to potato stem infection caused by *R. solani*. This implicates that these organisms should be indigenous in the soil. Soil disinfection with aldicarb increased the disease severity significantly in Experiment 1, but not significantly in Experiment 2. This positive effect of aldicarb on the disease severity indicates that there was a fauna in the soil suppressive to *R. solani*. Hofman (1988) stated that *F. fimetaria* and *A. avenae*, and to a smaller extent *T. krausbaueri*, are the most important mycophagous soil animals that lead to a reduction of Rhizoctonia stem canker of potato in fields. However, nematodes of the genus *Aphelenchoides* are also considered as important feeders on fungal pathogens (Curl, 1988). In our experiments, these four species were the dominant mycophagous springtails and nematodes and their populations were reduced by aldicarb.

Numbers of mycophagous animals in the soil were increased by organic soil amendments. Highest population densities of springtails were obtained with August application of FYM followed by white mustard as green manure crop. Sievers & Ulber (1990) also found dense populations of springtails with early (September) incorporation of straw into the soil followed by a green manure crop, but not after incorporating straw alone in November. A stimulative effect of FYM on mycophagous springtails was also found by Van de Bund (1970) and Curry & Purvis (1982). The same treatments that increased numbers of mycophagous springtails also enhanced the populations of non-parasitic nematodes, but probably the populations of mycophagous nematodes had not increased. Of the green manure crops, oats evidently stimulated the numbers of mycophagous nematodes. Although this crop did not affect the total numbers of mycophagous

springtails, the population of *F. fimetaria* increased.

The increased populations of mycophagous soil animals by organic amendments was associated with less Rhizoctonia stem canker in Experiment 1, but this association was weak in Experiment 2. In Experiment 2, only oats significantly reduced the disease severity. Lootsma & Scholte (Chapter 5) showed that adding organic rape material to the soil increased the population density of the nematode *A. avenae*, resulting in an increased suppressive effect on *R. solani*.

Stem infection by *R. solani* was much more severe in Experiment 2 than in Experiment 1. This probably explains the small effects of mycophagous animals on Rhizoctonia stem canker in Experiment 2. When inoculum is abundant, mycophagous animals probably fail to control *R. solani* effectively: there is so much inoculum compared to the numbers of animals that an important part of the fungus does not come into contact with the mesofauna and may cause plant infections. This supposition is supported by experiments carried out in growth chambers by Lootsma & Scholte (1997a) and by experiments of Voland & Epstein (1994). Voland & Epstein found that soil amendments with manure and compost reduced Rhizoctonia damping-off of radish at low inoculum levels of *R. solani*, but neither amendment was effective at high inoculum levels. It also explains why the effect of aldicarb on stem canker was not significant in Experiment 2. However, such severe soil infestations of *R. solani* are not very common in the Netherlands. Scholte (1992) found in crop rotation experiments on sandy soil, marine clay or reclaimed moor, in crop rotations with 50% potato, infection levels comparable to or lower than that of Experiment 1.

The much higher infection levels in Experiment 2 compared with Experiment 1 can be the result of various causes. Harvested tubers in August/Year 1 of Experiment 1 showed sclerotia severely infested by the antagonist *Verticillium biguttatum*, probably due to the relatively high soil temperature in that year. Probably, soil inoculum was partly killed by the antagonist. Moreover, Rhizoctonia stem infection can also be enhanced by the dry soil conditions in the spring of 1994 (Hide & Firmager, 1989). Seed tubers used in Year 1 of Experiment 2 to infest the soil were possibly infested with a more virulent strain of *R. solani* than in Experiment 1. We surmise that the use of seed tubers from different cultivars (Santé in Exp.1 and Spunta in Exp.2) to introduce R. solani did not affect soil infestation (Buhr, 1989). It is very unlikely that the difference in preceding crops (sugar beet in Exp. 1 and maize in Exp. 2) between the experiments had any effect on stem infections in Year 2 of the experiments (Scholte, 1992).

Under dry soil conditions mycophagous nematodes seem more suppressive than under wet soil conditions (Lootsma & Scholte, 1997b). Maybe, this explains why in Experiment 2 the

disease severity was lower after oats as green manure crop compared with the other treatments. Population density of mycophagous nematodes was by far the highest after oats.

There is no simple relation between application of organic soil amendments, population densities of mycophagous soil animals and Rhizoctonia stem canker for the following reasons: a) the mycophagous soil fauna consists of a complex of varying animals and the animals are not equally suppressive to *R. solani* (Hofman *et al.*, 1990), b) organic material not only stimulates populations of mycophagous soil organisms but also the saprophytic activity of *R. solani* (Moubasher & Abdel-Hafez, 1986), c) alternative food sources can reduce the grazing activity of springtails on *R. solani* (Lootsma & Scholte, Chapter 5), d) metabolites of decomposing organic material affect activities of soil organisms (Mojtahedi *et al.*, 1993) and e) organic material can stimulate the soil flora antagonistic to *R. solani* (Wu, 1986).

The results support the hypothesis that increasing the activity of mycophagous soil animals in the soil can contribute to a reduction in Rhizoctonia disease. However, further experimentation is needed. The experiments showed evidently that populations of mycophagous soil animals can be enhanced by organic amendments. August incorporation of FYM into the soil followed by oats as green manure crop could be a good combination, but was not tested.

Chapter 7

General Discussion

Chapter 7

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Soil-borne inoculum of *R. solani* is responsible for serious yield and quality losses in potato. Sprouts of emerging potato plants are infected resulting in weaker and fewer stems per plant. Later stolons and young tubers are also seriously infected. At the end of the growing season sclerotia are formed for survival on tubers and subterranean plant debris (stems, stolons and roots). Controlling the build-up of soil inoculum can contribute to fewer plant infections in the next potato crop on the same field.

Effects of harvesting methods

Formation of sclerotia on tubers can be affected by harvesting methods. Especially green-crop-harvesting (Mulder *et al.*, 1992) and, to a smaller extent, haulm pulling reduce the formation of sclerotia on tubers, whereas haulm killing by chemicals stimulates the formation of sclerotia (Dijst, 1990).

Our experiments showed that soil infestation can also be affected by harvest methods. Soil infestation was estimated on the basis of stem and stolon infections of potato in the subsequent year. Haulm pulling had no effect at all compared with chemical haulm killing. Immature-crop-harvesting, effective to avoid black scurf (sclerotia) formation on progeny tubers, also lowered the disease severity in the next crop. However, this harvest method was only successful in controlling the disease when the formation of sclerotia did not start before harvest and the crop debris was incorporated into the soil with a rotary hoe. Dijst (1990) stated that volatile and unstable exudates from potato tubers stimulate the formation of sclerotia. This is possibly also true for root exudates. Sufficient aeration of the soil reduces the stability and accumulation of these components. Cultivation with the rotary hoe clearly resulted in better soil aeration. When haulm debris (aerial stems and leaves, below-ground stems and stolons) was incorporated into the soil, the disease severity was lower than when the debris was left on the soil surface or completely removed from the field. This indicates that plant roots are also important for the formation of sclerotia. Tillage of the soil with a rotary hoe improves soil aeration but also destroys the root system, probably resulting in lower levels of root exudates and less stimulation of sclerotia formation. However, a contrasting mechanism may explain the effect of incorporating

haulm debris because saprophytic growth of *R. solani* can be enhanced after incorporation. Incorporation can stimulate natural antagonists (both mycophagous soil fauna and mycoparasites), ultimately resulting in a lower level of *R. solani.*

Thus, immature-crop-harvest when applied before formation of sclerotia, followed by soil tillage with a rotary hoe, can reduce disease severity in the subsequent potato crop, very probably because of a lower soil infestation level.

Mycophagous soil fauna

Evaluation of techniques used under controlled conditions

Another way to reduce stem and stolon infections of potato plants may be obtained by the use of antagonists. Hofman (1988) showed that the mycophagous mesofauna reduces infections of potato by grazing *R. solani* mycelium or by sucking out the contents of the hyphae. The efficacy of these animals under various environmental conditions was investigated in growth chambers using a special experimental set-up. This method appeared to be satisfactory. Within experiments, the variation of *R. solani* soil infestation could be minimized to the extent that the antagonistic effects of the soil fauna were measurable. Discrepancy between results of replicate experiments can be partly attributed to insufficient standardization of conditions during the period of incubation. Especially variations in soil moisture are important and can affect the multiplication rate of soil fauna after introduction to the soil. Also the method used to introduce the animals to the soil may have caused additional variation. The organisms were applied to the system as a mass culture with animals of different ages, ranging from the egg stage to adults. This agrees with the situation in the field but may have caused greater variation. The mass culture also contained food sources (*Alternaria porri*) for the mycophagous soil fauna. The amount of extra food that was added together with the animals was difficult to control and may have differed between experiments.

The experimental set-up was designed to provide an incubation period without disturbing the soil after inoculation with *R. solani* and mycophagous animals before seed tubers were planted. This method can also be used to investigate the population dynamics of *R. solani* and various antagonists over a longer period than was done in the present experiments. The method is suitable for further studies on the population dynamics within a tritrophic system.

Results of experiments under controlled conditions

The growth chamber experiments showed that the mycophagous soil fauna is effective in

reducing Rhizoctonia stem canker both at 10 °C and at 15 °C. This finding is very important because in the Netherlands potatoes are planted from approximately mid-March until early May, when soil temperatures range from 8 to 13 °C. *R. solani* is already active at temperatures lower than 10 °C (Van den Boogert & Jager, 1984). At temperatures of 10 to 15 °C, infection of potato by *R. solani* is most severe (Hide & Firmager, 1989). *R. solani* infection is also most severe under dry soil conditions (Hide & Firmager, 1989; Lootsma & Scholte, 1997b). Our results showed that under wet conditions springtails were most effective and under dry conditions the mycophagous nematodes were more effective. This indicates that the control of *R. solani* by a complex of different organisms may be robust over a range of environmental conditions.

The growth chamber experiments also showed that adding dried rape material to the soil can have various effects on the suppressive potential of mycophagous organisms, depending on the composition of the organic material and the soil pH. The ultimate effect is caused by a complex of interactions between the organic material, the environmental conditions, the saprophytic flora and fauna, *R. solani* and the mycophagous soil fauna. Within this complex, the direct effect of organic material on *R. solani* plays an important role. Organic material can stimulate the saprophytic growth of *R. solani* in some cases and this reduces the net result of suppression by mycophagous soil animals. High populations of springtails did not reduce *R. solani* under all circumstances. In the growth chamber experiments, fresh organic material was applied to the soil. However, although numbers of mycophagous animals were equal or higher compared with untreated soil, their controlling effect on *R. solani* decreased, especially at high soil pH, probably due to the presence of alternative food sources. Organic material applied in the absence of mycophagous animals had no or only a small positive effect on Rhizoctonia stem infection.

Results of field experiments

In the field experiments, one year old farmyard manure was incorporated into the soil in mid-August or debris of died (by frost) green manure crops was incorporated mid-March. Most likely, easily degradable components of the material were already decomposed when potato was planted mid-April. This may explain, that, if organic material was incorporated into the soil there was a better correlation between numbers of mycophagous soil animals and stem and stolon infections of potato in the field experiments than in the growth chamber experiments. However, the relations between the type of organic material, its state of degradation, and the activity of the mycophagous animals need further research. Further research is also needed on the effect of the spatial distribution of organic matter in the soil. In the field experiments organic matter was

incorporated into the soil with a spading machine to distribute the material evenly through the soil. The organic material will be distributed less evenly through the soil after ploughing.

The field experiments showed that the suppressive potential of the mycophagous soil fauna can be manipulated in such a way that stem canker can be reduced. The use of oats as a green manure crop looks especially promising because it stimulates both mycophagous nematodes and the proportion of effective springtails, and reduces stem infections.

Evaluation of the bio-ecological approach

Can addition of organic amendments to the soil reduce stem canker to an acceptable level? The results of the field experiments and the growth chamber experiments do not give a conclusive answer to this question, however, the following remarks can be made. In Field experiment 1 (Chapter 6) soil infestation was comparable with the level that can be expected in farmers' fields (Scholte, 1992). In this experiment, Rhizoctonia was suppressed to low levels. In Field experiment 2 (Chapter 6) soil infestation was unrealistically high. The fact that oats as a green manure crop still reduced stem canker under these circumstances indicates that there is a strong active mechanism that reduces stem canker. The results of the field experiments also indicate that further optimization is possible. Reduction of infection when white mustard was used as a green manure crop was improved when it was combined with the application of farmyard manure at the date of sowing. Springtail populations were very high for this combination. It can be expected that the combination of oats with farmyard manure will result in similar or even greater effects. This would mean that both the springtails and the mycophagous nematodes are stimulated and that there will be a strong reduction of stem canker. The reducing effect of mycophagous soil animals on Rhizoctonia stem infections can be improved by combining it with other biological methods. The mycoparasite *Verticillium biguttatum* has proven to be an efficient controlling agent of *R. solani* at higher soil temperatures. There are no indications that the mycophagous soil fauna reduces the density of *V. biguttatum.* None of the organic treatments in the field experiments reduced *V. biguttatum* on black scurf in the field experiment (M. Lootsma, unpublished data) although the populations of mycophagous soil organisms were very high. The growth of an appropriate green manure crop in autumn combined with treatment of seed tubers with *V. biguttatum* could result in a pool of organisms able to suppress *R. solani* over a wide range of temperatures.

The above described bio-ecological approach of stem canker control has some important advantages compared with the classical biological control where one specific antagonist or

competitor is introduced into the system. The bio-ecological approach is based on optimization of a suppressive biological complex that is already present in the soil. This means that the problems that are often accompanied with the introduction of a biological agent into the soil are not present. The antagonists used in this system have already adapted to the extreme conditions in a potato field, which is characterized by a high degree of soil disturbance. The suppressive capacity is not based on one species but on a complex of mycophagous soil organisms: various species of springtails, nematodes and also mites (Enami & Nakamura, 1996). This means that the range of environmental conditions with suppressive potential is broader than with a system based on one antagonist. The above mentioned suppression of *R. solani* under wet and dry soil conditions by different mycophagous soil organisms is a clear example of this principle. The indigenous nature of these animals makes it possible to use other food resources for reaching high populations. This is an important advantage compared with the mycoparasite *V. biguttatum*, which is dependent on the presence of *R. solani* in the soil. A problem with application of a specific biological agent is often placement at the right site of action. In contrast, mycophagous soil animals tend to aggregate on or close to the surface of underground plant organs, exactly where they are needed to prevent *R. solani* infections. They also aggregate more under dry soil conditions (Curl & Truelove, 1986) when *R. solani* is more harmful to potato (Hide & Firmager, 1989).

The finding that application of granular nematicides/insecticides increases Rhizoctonia stem infection in various soil types (Scholte, 1987; Hofman, 1988) indicates that the mycophagous soil fauna is present in different soils, although the composition of the complex may differ with soil type. Therefore, it may be expected that manipulation of the suppressive soil fauna can also be achieved in other soil types than only a sandy soil. In modern farming systems there is an increasing need to include green manure crops in the cropping systems because of the positive effects on soil fertility, prevention of nitrogen leaching (nutrient catch crops), and avoiding wind and water erosion. The growth of the appropriate green manure crop in the year before potato is grown, combined with an application of farmyard manure, can contribute to control of Rhizoctonia stem and stolon canker.

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Summary

Rhizoctonia solani (AG-3) is a soil-borne plant pathogen causing canker on potato stems and stolons. Severe canker results in delayed emergence, fewer emerged stems, lower tuber yield, and a high proportion of small, partly green and misshapen tubers with a low dry matter content. The pathogen survives for several years in the soil by sclerotia. Sclerotia can also be formed on progeny tubers (black scurf). In this thesis two approaches for non-chemical control of stem canker, caused by soil-borne inoculum of *R. solani*, are investigated.

Sclerotia of *R. solani* are formed on progeny tubers and other below-ground plant parts at the end of the growing season. The levels of black scurf on tubers can be affected by harvest methods (such as chemical haulm destruction, green-crop-harvesting, and haulm pulling) that are used in seed potato production in the Netherlands. Two field experiments were conducted to investigate whether these harvest methods also affect soil infestation. Soil infestation was estimated on the basis of stem and stolon infestations in the subsequent year. The effect of harvest methods depended on soil infestation level at immature harvest time. Immature-crop-harvesting lowered the disease severity in the next crop compared with haulm pulling and chemical haulm killing. However, this harvest method was only successful in controlling the disease when the formation of sclerotia did not start before harvest and the crop debris was incorporated into the soil with a rotary hoe.

Rhizoctonia stem and stolon canker can also be reduced by the mycophagous soil mesofauna. Springtails and nematodes feed directly on the mycelium of *R. solani*. These animals are indigenous in arable field soils. A two-year-lasting field experiment was conducted twice to assess the effects of green manure crops and farmyard manure on Rhizoctonia stem canker and the populations of mycophagous soil organisms.

In the first year the field was infested with *R. solani* by growing a potato crop from severely infested seed tubers. After harvesting the crop in August, farmyard manure was incorporated into the soil and/or various green manure crops were grown. In the subsequent year population densities of mycophagous soil animals and stem infection by *R. solani* on potato were assessed. Oats grown as a green manure crop or farmyard manure plus white mustard as green manure crop enhanced the populations of the mycophagous soil fauna and reduced the severity of Rhizoctonia stem and stolon canker. Oats especially increased the populations of mycophagous nematodes, whereas farmyard manure plus white mustard mainly enhanced the populations of mycophagous springtails. Effects of environmental factors on the suppression of

Rhizoctonia stem canker by the springtail *Folsomia fimetaria* and the mycophagous nematode *Aphelenchus avenae* were investigated under controlled conditions. Low soil temperatures and relatively dry soil conditions are favourable for Rhizoctonia stem canker, however, the mycophagous animals were very suppressive under these conditions. When fresh organic rape material was applied to the soil under controlled conditions, populations of *F. fimetaria* and *A. avenae* were mostly higher than in untreated soil. However, the controlling effect of *F. fimetaria* on *R. solani* decreased, especially at high soil pH, probably due to the presence of alternative food sources.

Samenvatting

Rhizoctonia solani (AG-3) is een bodemschimmel die ernstige schade kan toebrengen aan het gewas aardappel. De schimmel veroorzaakt vooral in het voorjaar lesies op de ondergrondse stengeldelen en stolonen. Bij een zware aantasting kan dit zelfs leiden tot het afsterven van deze plantorganen. Schade aan het gewas komt tot uiting in een verlate opkomst, minder stengels per poter, lagere knolopbrengsten, een groter aandeel kleine en gedeeltelijk groene, vaak misvormde knollen en lagere zetmeelgehaltes. Overlevingsstructuren (sclerotiën) van de schimmel kunnen zowel op de knollen (lakschurft) als in de bodem worden gevormd.

Het doel van het onderzoek dat in dit proefschrift is beschreven was het verkennen van niet-chemische teeltmaatregelen die kunnen leiden tot een betere beheersing van stengel- en stoloninfecties door *R. solani*. Het ging daarbij om infecties vanuit de bodem in het voorjaar tijdens de opkomst en de eerste groeifase van een aardappelgewas. Er werd gekozen voor twee benaderingen: 1) onderzoek naar mogelijkheden om de bodembesmetting met *R. solani* na de teelt van een aardappelgewas te verlagen; 2) onderzoek naar mogelijkheden om de onderdrukking van *R. solani* door de mesofauna in de bodem te versterken.

Verlaging van de bodembesmetting met *R. solani* zou kunnen worden gerealiseerd door de wijze van oogsten van een aardappelgewas. De vorming van overlevingsstructuren van *R. solani* op aardappelknollen in het najaar is sterk afhankelijk van de behandeling van het aardappelgewas voor en tijdens de oogst. Twee tot drie weken voor de oogst doodspuiten van nog niet afgerijpt loof leidt tot een hogere lakschurftbezetting op de knollen dan wanneer het loof twee tot drie weken voor de oogst wordt getrokken (looftrekken). De laagste aantallen sclerotia komen op de knollen voor wanneer de knollen gelijktijdig met het trekken van het loof worden geoogst (het groenrooien). In twee veldproeven werd onderzocht in hoeverre dergelijke uiteenlopende oogstmethoden van een aardappelgewas ook een effect hadden op de bodembesmetting met *R. solani*. De bodembesmetting werd gemeten door in het jaar, dat volgde op de toepassing van de verschillende rooimethoden, nog een keer aardappelen te telen als testgewas, waarbij de mate van stengelaantasting van dit testgewas werd gehanteerd als een indirecte maat voor de bodembesmetting met *R. solani*. Op deze manier werd de invloed van doodspuiten, looftrekken, groenrooien en rijp rooien (na natuurlijke afrijping van het gewas) op de stengelaantasting in het volgende jaar bepaald. Alleen groenrooien bleek de bodembesmetting te reduceren mits het plaatsvond wanneer de lakschurftvorming op de knollen nog niet of in zeer beperkte mate was

begonnen en mits gewasresten na de oogsthandeling direct in de grond werden gewerkt met een langzaam draaiende frees.

Versterking van de onderdrukking van *R. solani* door de fungivore mesofauna zou kunnen worden bereikt door beïnvloeding van de populatieomvang en de effectiviteit van deze organismen. Vooral bepaalde springstaarten en aaltjes zijn in staat om zich direct te voeden met schimmeldraden van *R. solani*. Deze diertjes komen algemeen voor in akkerbodems. Effecten van omgevingsfactoren op de potentiële onderdrukking van een Rhizoctonia stengelaantasting door de springstaart *Folsomia fimetaria* en het aaltje *Aphelenchus avenae* werden onderzocht in klimaatcellen. Beide organismen gelden als belangrijke representanten van de fungivore bodemfauna. Voor deze proeven in klimaatcellen werd een speciale proefopstelling ontwikkeld. Beïnvloeding van de populatieomvang van fungivore organismen om een betere onderdrukking van *R. solani* te bewerkstelligen werd onderzocht door middel van proeven in klimaatcellen en in het veld.

Uit de experimenten in klimaatcellen bleek, dat de fungivore bodemfauna ook bij een lage bodemtemperatuur (10 °C) en zowel onder vochtige als droge bodemomstandigheden effectief de stengelaantasting door *R. solani* kon onderdrukken. Onder droge omstandigheden waren met name fungivore aaltjes effectief. Uit proeven in klimaatcellen bleek ook, dat toevoeging van gedroogde bladkool aan de grond de populatieomvang van zowel fungivore springstaarten als fungivore aaltjes deed toenemen. Materiaal afkomstig van oude bladkoolplanten was daarbij effectiever dan materiaal afkomstig van jonge planten. De toevoeging van organisch materiaal aan de grond leidde evenwel niet altijd tot minder Rhizoctonia stengelinfecties. Het onderdrukkend effect van springstaarten ging bijna geheel verloren wanneer het organisch materiaal werd toe-gevoegd aan een grond met een pH van 6.0 vergeleken met toediening aan een grond met een pH van 4.8. Dit afgenomen onderdrukkend effect ten opzichte van *R. solani* werd waarschijnlijk veroorzaakt door een groter aanbod van alternatieve voedselbronnen (niet pathogene schimmels en bacteriën, organisch materiaal) voor de mesofauna.

In twee veldproeven (Experimenten 1 en 2) werd met verschillende vormen van organische bemesting (stalmest en/of de teelt van groenbemestingsgewassen) getracht de populatiedichtheden van de fungivore bodemorganismen in de bodem te verhogen teneinde de stengelinfectie met Rhizoctonia te verlagen. In beide proeven werden inderdaad met organische bemestingen hogere aantallen fungivore bodemorganismen waargenomen. Het grootste positieve effect op de springstaartpopulatie werd bereikt wanneer half augustus 35 ton stalmest/ha in de grond werd gewerkt en daarna gele mosterd werd geteeld als groenbemestingsgewas. Wanneer haver als

groenbemestingsgewas werd geteeld (zonder stalmest) nam vooral de populatie van fungivore aaltjes zeer sterk toe. In Experiment 1, met een niveau van Rhizoctonia stengelinfecties zoals die in Nederland meestal worden aangetroffen in rotaties met een hoge teeltfrequentie van aardappelen, leidden de organische bemestingen tot lagere Rhizoctonia stengelinfecties. In Experiment 2 was de stengelaantasting echter zo hevig, dat de opkomst van de aardappelplanten zeer onregelmatig was; sommige planten kwamen zelfs helemaal niet op of stierven kort na opkomst. Hoewel de trends van de effecten van organische bemestingen op de Rhizoctonia-aantasting meestal gelijk waren aan die in Experiment 1, waren de effecten niet significant, behalve wanneer haver als groenbemester werd geteeld. De resultaten ondersteunen de hypothese, dat met behulp van organische bemesting de populatieomvang van fungivore bodemorganismen kan worden verhoogd met als resultaat dat de stengel- en stoloonaantasting door *R. solani* kan worden onderdrukt.

Curriculum vitae

Metske Lootsma werd geboren in Drachten op 15 juli 1959. Na het behalen van het Gymnasium diploma aan het Gereformeerd Lyceum te Groningen begon hij in 1977 met de studie Landbouwplantenteelt aan de toenmalige Landbouwhogeschool te Wageningen. In 1986 studeerde hij af met de vakken Landbouwplantenteelt, Entomologie en Onkruidkunde in zijn doctoraalpakket. In de periode van 1987 tot 1990 werkte hij achtereenvolgens als proefveldmedewerker, onderzoeksassistent en als onderzoeker bij een plantenveredelingsinstituut dat aanvankelijk SVP heette en daarna via CPO veranderde in het CPRO. Hij verrichtte daar onderzoek in de gewassen aardpeer, maïs en grassen. Vanaf 1 januari 1991 was hij 4 jaar in dienst bij de vakgroep Agronomie van de Landbouwuniversiteit als assistent in opleiding. Tijdens deze periode werd het onderzoek uitgevoerd dat beschreven is in dit proefschrift. Van maart 1996 tot augustus 1997 was hij werkzaam als docent natuur- en scheikunde aan achtereenvolgens het Greydanus College te Zwolle en het Willem van Oranje College te Waalwijk.
Per 1 december 1997 heeft hij een betrekking bij Profuse B.V. te Veenendaal.