

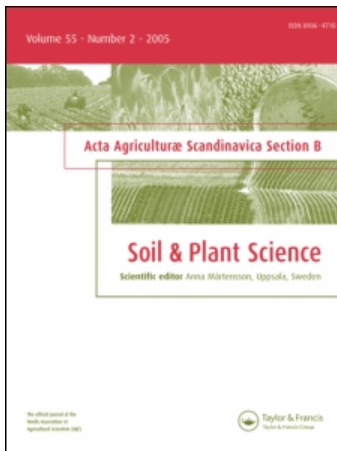
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ORIGINAL ARTICLE

Effects of fungivorous nematodes on corky root disease of tomato grown in compost-amended soil

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Abstract

The effect of fungivorous nematodes, *Aphelenchus avenae* and *Aphelenchoides* spp., against corky root disease of tomato caused by *Pyrenochaeta lycopersici* was investigated. Three different greenhouse trials were conducted using soil naturally infested with *P. lycopersici*, alone or mixed with four different types of compost consisting of green manure, garden waste and horse manure (20% compost by volume). The fungivorous nematodes were propagated in cultures of the fungus *Pochonia bulbillosa* and inoculated (3 or 23 nematodes ml⁻¹ substrate) into the soil and soil-compost mixtures one day after transplanting of tomato seedlings. Greenhouse experiments were terminated after ten weeks and disease was measured from infected roots after harvesting. *Aphelenchus avenae* significantly reduced the disease severity when added to infested soil without compost in all experiments. *Aphelenchoides* spp. did not suppress the disease either in the presence or absence of compost. Among the composts tested, only a garden waste compost was found to be suppressive to the disease. Neither *A. avenae* nor *Aphelenchoides* spp. improved the suppressive effect of the compost.

Keywords: *Aphelenchus avenae*, *Aphelenchoides* spp., biological control, compost, *Pyrenochaeta lycopersici*, tomato.

Introduction

Biological control is an attractive approach for management of soil-borne diseases. The benefits of biological control for human health and the environment have resulted in increased attention to this approach in controlling crop diseases, especially in greenhouse crop production systems. The controlled environment of the greenhouse offers a unique niche for the biological control of soil-borne plant diseases (Paulitz & Bélanger, 2001). Research has shown that the use of beneficial fungi, bacteria or fungivorous nematodes can be effective in the control of soil-borne fungi in the greenhouse (Barnes et al., 1981; Datnoff et al., 1995; Arndt et al., 1998).

Corky root disease (causal agent *Pyrenochaeta lycopersici* Schneider & Gerlach) is a serious problem when growing tomatoes in soil substrates. The current control methods for corky root disease management have various limitations. For example, use of grafted tomato plants (grafting a commercial variety to a rootstock tolerant to *P. lycopersici*) is an

effective method but it greatly increases planting costs and may impair the taste of tomatoes depending on the rootstocks and grafted varieties. Tolerant rootstocks can also be attacked when the inoculum level of *P. lycopersici* in the soil is high. Inoculum levels in infested soil can be reduced by steaming or solarization (Last & Ebben, 1968; Moura & Palminha, 1994; Ioannou, 2000). Soil solarization is suitable for countries having a sunny climate. Steaming is quite costly, it may negatively affect the whole soil biota and there is also a risk that inoculum of *P. lycopersici* can be left in deeper soil layers due to limited steam penetration. These difficulties in controlling corky root disease by available methods provide good reason for developing new biological controls for corky root disease.

Trichoderma spp., well-known fungal antagonists, have shown promising results against *P. lycopersici* in *in vitro* experiments (Whipps, 1987; Vanachter et al., 1988). In a large-scale greenhouse trial, Bochow (1989) used a bacterial antagonist, *Bacillus subtilis* T99, against *P. lycopersici* and reported considerably

lower disease severity and 5% higher yield in the treated plots than in the untreated plots.

Fungivorous nematodes are equipped with a mouth stylet, which they use to penetrate fungal cells, withdraw the cell contents and thus kill the cells (Yeates et al., 1993). *Aphelenchus avenae* Bastian and *Aphelenchoides composticola* Franklin are widely distributed fungivorous nematodes that have a biological control potential for certain plant parasitic soil fungi like *Fusarium*, *Pythium*, *Rhizoctonia* (Gupta, 1986; Ishibashi & Choi, 1991; Okada, 2006). *Aphelenchus avenae* has been shown to control root rot of corn, caused by *Pythium arrhenomanes* Drechsler, under greenhouse conditions (Rhoades & Linfood, 1959). Klink and Barker (1968) found that *A. avenae* could inhibit the formation of new sclerotia of *Rhizoctonia solani* Kühn. Damage to wheat caused by *Fusarium culmorum* (W.G. Smith) Saccardo and *Gerlachia nivalis* W. Gams & E. Muller was significantly reduced by *Aphelenchoides* spp. in pot trials under controlled conditions (Rössner & Urland, 1983). The effect of fungivorous nematodes on corky root disease has not previously been investigated.

Compost has also been reported to protect plants from soil-borne root pathogens when used as a soil amendment or in container media (Hoitink & Fahy, 1986; Chen et al., 1987; Diab et al., 2003; Noble & Coventry, 2005; Termorshuizen et al., 2006). Disease suppression has often been related to biotic rather than abiotic factors of the compost (Reuveni et al., 2002). Microbial communities in the compost can suppress the pathogen by competition, antibiosis, parasitism/predation and systemic induced resistance of the host plant (Hoitink & Boehm, 1999). Pathogen-specific antagonists cannot always be expected to be present in the early stage of composting. Therefore, it is recommended that specific antagonists should be added to the compost to increase the disease suppression activity (Segall, 1995). Composts amended with specific antagonists can be up to three times as suppressive as unamended, naturally suppressive composts (Nelson et al., 1983; Cotxarrera et al., 2002; Postma et al., 2003).

In the present study, we conducted greenhouse trials in which soil naturally infested with *P. lycopersici* was used to grow tomato plants and the separate and combined effects of compost amendment and fungivorous nematodes on corky root disease were evaluated. Our hypotheses were: 1) compost amendment and *A. avenae* and *Aphelenchoides* spp. would decrease corky root disease severity; 2) the disease suppressive effect of the compost would increase with fungivorous nematode enrichment.

Materials and methods

Soil and compost

The infested soil was collected from an organic tomato grower's greenhouse in the vicinity of Uppsala (59°49'N, 17°43'E), Sweden. Four different types of compost were used: 1) green manure compost (GMC) made from red clover mixed with 10–20% (d.w.) chopped straw; 2) horse manure compost (HMC) made from unknown proportions of horse manure and peat; 3) garden waste compost 1 (GC1) made from 70% (vol.) garden waste and 30% (vol.) horse manure with an unknown proportion of straw; and 4) garden waste compost 2 (GC2) made from 70% (vol.) garden waste and 30% (vol.) horse manure with an unknown proportion of peat. All the composts originated from Sweden. For organic tomato growers in Sweden, it is possible to produce green manure compost or horse manure compost on farms from locally available resources or buy garden waste composts from a commercial composting plant. The soil and composts were stored in a cold room at 4°C prior to use.

Sources and cultures of fungivorous nematodes

Aphelenchus avenae was isolated from a potato field soil in Västergötland, Sweden (approx. 58°10'N, 13°34'E). *Aphelenchoides* spp. were isolated from a compost coded 'm' (wood chips 88%, manure 2.5% and clay 10%) originating in the Netherlands (Termorshuizen et al., 2006). In the experiments, a mixture of two *Aphelenchoides* species isolated from the compost was used and is referred to here as *Aphelenchoides* spp. In the laboratory, *A. avenae* and *Aphelenchoides* spp. were propagated in cultures of the fungus *Pochonia bulbillosa* W. Gams & Malla growing in malt extract bacto agar (Difco Lab, USA). The initial cultivation of nematodes was successfully accomplished by selecting specimens from nematode samples extracted from the soil. These nematodes were surface sterilized by chlorhexidine 0.5% for 20 min and repeatedly rinsed in sterile water. Thereafter, 5–10 sterilized nematodes in 50 µl sterile water were placed in a hole (0.5 cm diameter) in the centre of agar plates with *P. bulbillosa* (5 cm diameter). After four weeks, nematodes covered the whole plates. Subsequently, for mass propagation, two 1.5-cm diameter pieces of agar from these cultures with *P. bulbillosa* and nematodes were placed in two opposite holes from the margin of fungal colonies in other monoxenic cultures of *P. bulbillosa* on agar plates (9 cm diameter). The plates were sealed and placed in the dark at room temperature (20–22°C) for four weeks

for nematode propagation. The cultures were subsequently stored at 5°C.

Characterization of initial nematode population

To determine the initial population of nematodes in the infested soil, composts and soil-compost mixtures, nematodes were extracted by the Baermann funnel method (Southey, 1986) for 24 h at approximately 20°C. For each type of substrate, four replicates (30 ml each) were taken. Nematodes were counted under a stereomicroscope at 25–50 × magnification and categorized into one of four groups: 1) *Aphelenchus avenae*, 2) *Aphelenchoides* spp., 3) other fungivorous nematodes, 4) bacterivorous and other (non-fungivorous) nematodes.

Greenhouse experiments

Three different experiments were carried out in the greenhouse located in Uppsala, Sweden: experiments 1 and 2 in 2004 and experiment 3 in 2005. All experiments were conducted in the same infested soil, referred to here as IS1 (infested soil used in 2004) and IS2 (infested soil used in 2005). The infested soil was stored at 4°C between the two consecutive years. The various composts were mixed with the infested soil at a rate of 20% (vol.) as compost inclusion rates of at least 20% (v/v) have been reported optimal for disease suppression (Noble & Coventry, 2005). The following treatments were included: experiment 1: i) IS1, ii) IS1 + green manure compost (GMC), iii) IS1 + *A. avenae* and iv) IS1 + GMC + *A. avenae*. Experiment 2: i) IS1, ii) IS1 + garden waste compost 2 (GC2), iii) IS1 + *A. avenae*, iv) IS1 + *Aphelenchoides* spp., v) IS1 + GC2 + *A. avenae* and vi) IS1 + GC2 + *Aphelenchoides* spp. Experiment 3: i) IS2, ii) IS2 + horse manure compost (HMC), iii) IS2 + garden waste compost 1 (GC1), iv) IS2 + *A. avenae*, v) IS2 + *Aphelenchoides* spp., vi) IS2 + HMC + *A. avenae*, vii) IS2 + HMC + *Aphelenchoides* spp., viii) IS2 + GC1 + *A. avenae*, ix) IS2 + GC1 + *Aphelenchoides* spp. Nematode inoculation rate was 3 nematodes ml⁻¹ substrate in experiment 1 and 23 nematodes ml⁻¹ substrate for *A. avenae* and 33 nematodes ml⁻¹ substrate for *Aphelenchoides* spp. in experiments 2 and 3. The inoculation rate was lower in experiment 1 than in experiments 2 and 3 because of a lower production of nematodes in the mass culture at the beginning of the experiments. However, in experiment 3, low inoculation rate of *A. avenae* and *Aphelenchoides* spp. in the infested soil (3 nematodes ml⁻¹ substrate) was included as two more treatments to compare with the effects of high inoculation rates (23 and 33 nematodes ml⁻¹ substrate for *A. avenae* and

Aphelenchoides spp., respectively). A fully randomized design was followed in the greenhouse experiment. In experiment 1 there were three replicates for nematode inoculated treatments and ten for non-inoculated treatments, in experiment 2 there were six replicates for nematode inoculated treatments and eight for non-inoculated treatments, and in experiment 3 there were eight replicates for all treatments. Due to lack of sufficient number of nematodes, the same number of replicates was not used in all experimental treatments.

Tomato seeds (*Lycopersicon esculentum* cv. Elin, Weibulls®, Sweden) were sown in commercial soil (Hasselfors Garden E-Jord®, Sweden). Three-week-old seedlings were transferred to plastic pots with 5 litres soil or soil-compost mixture in the greenhouse. Each pot contained a single seedling. Nematodes were inoculated into the pots one day after transplanting of tomato seedlings. Nematodes grown on fungus cultures in agar medium were extracted by the method mentioned earlier. Nematode density in the extract was adjusted by adding tap water so that 30-ml suspension in each pot gave 3 or 23 or 33 nematodes ml⁻¹ growing substrate. Six holes (5 cm depth) were made around a seedling and 5 ml of nematode suspension were added to each hole, after which the holes were immediately covered with soil.

The plants were irrigated twice a day with normal tap water. To avoid nutrient deficiency of the tomato plants, organic fertilizer was applied to all treatments when the plants were eight-weeks-old. The average temperature during the experiments varied from 18°C to 22°C and relative humidity was maintained at 70%. Artificial light was used only in greenhouse experiment 2 for 16 h per day for one month before harvesting because of short day length.

Harvest

The experiment was terminated ten weeks after transplanting of tomato seedlings since an earlier trial in the greenhouse on transplanting three-week-old tomato seedlings in pots with 5 litres infested soil showed that the plants required ten weeks to develop characteristic corky root disease symptoms on roots. During the experiment, fruits were picked as they ripened. At harvest, all fruits (green and red fruits) from each plant were weighed together. Total fruit weight was expressed as a cumulative weight. The shoots were cut off at the soil surface. The roots were separated from the soil by gentle shaking and rinsed with tap water to remove soil particles. Samples of 250 ml soil from each pot were taken for nematode counting and were kept in the cold room at 4°C until extraction. For extraction, 30-ml sub-samples from the 250 ml soil samples taken from each pot were

placed in a Baermann funnel for 24 h at approximately 20°C. Nematodes were counted and classified as described above. Fresh and dry weights of roots (after 24 h at 65°C) and shoots (after 48 h at 65°C) were measured.

Disease severity in each plant was evaluated by collecting the following three 3-cm sections of root sample: leaving a segment of 5 cm from the root base and then taking a 3-cm sample, leaving 5 cm and then taking another 3-cm sample, leaving 5 cm and then taking another 3-cm sample. The three root samples from three distances of each plant were pooled and mixed. From these root samples, 100 pieces from each plant were examined under a stereomicroscope and grouped into three categories as white (healthy root), light brown (initially infected root) and dark brown (severely infected root).

Population development of *Aphelenchus avenae*

In 2006, a greenhouse experiment was conducted to measure population development of *Aphelenchus avenae* in the same infested soil. Three-week-old tomato seedlings (cv. Elin) were transplanted into plastic pots with 5 litres infested soil. One day later, *A. avenae* was inoculated (23 nematodes ml⁻¹ substrate) into these pots using the procedure described earlier. The experiment continued for ten weeks in the same greenhouse conditions. Soil samples for nematode counting were collected 5, 10, 15, 20, 35 and 70 days after inoculation, with four pots being destructively sampled each time. Nematodes were extracted and counted as described above.

Statistical analysis

To model the probabilities of healthy, initially infected and severely infected roots, a generalized linear model for ordinal-scaled observations was fitted with the procedure GENMOD in SAS. The logit link was used and overdispersion within the root was modelled with the option DSCALE. The model was a factorial design with nematode and compost as main effects. CONTRASTS were used to separate different treatments. To determine the treatment effect on shoot and root dry weight and total fruit weight, ANOVA was performed in Minitab (version 14). Tukey's test was used as the multiple comparison method to compare the means. Nematode population data were analysed using ANOVA in Minitab (version 14) where treatment means were compared by Tukey's test. Data on nematode populations were log transformed prior to analysis.

Results

Effects of fungivorous nematodes and composts on corky root disease

Aphelenchoides spp. did not reduce corky root disease severity in the infested soil or in soil-compost mixtures in any of the experiments and therefore only the results with *Aphelenchus avenae* are presented. In experiment 1 (*A. avenae* inoculation rate 3 nematodes ml⁻¹ substrate), there was a significant interaction ($p=0.0071$) between green manure compost (GMC) and *A. avenae*. Soil amended with GMC alone had no effect on corky root disease reduction (Figure 1). *Aphelenchus avenae* significantly ($p=0.0068$) reduced disease severity when applied to infested soil without GMC. In combined application of GMC and *A. avenae*, the effect of the nematode was no longer significant.

In experiment 2 (*A. avenae* inoculation rate 23 nematodes ml⁻¹ substrate), there was no significant interaction between the factors compost and nematode. Garden waste compost 2 (GC2) had no suppressive effect on the disease. *Aphelenchus avenae* decreased the disease severity significantly ($p=0.0024$) in the infested soil in the absence of GC2 (Figure 2).

In experiment 3 (*A. avenae* inoculation rate 23 nematodes ml⁻¹ substrate), horse manure compost (HMC) greatly increased the disease severity (data not shown) and therefore we excluded HMC from further analysis. The effects of garden waste compost 1 (GC1) together with *A. avenae* are presented in Figure 3. There was a significant interaction ($p<0.0001$) between GC1 and *A. avenae*. GC1 showed significant ($p<0.0001$) disease reduction in the infested soil but its suppressive effect was

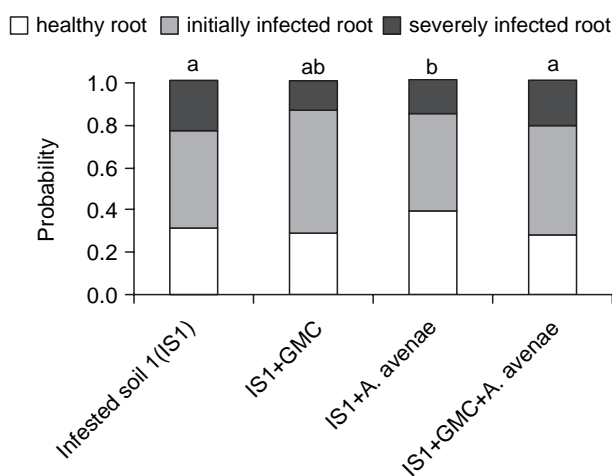


Figure 1. Effect of *Aphelenchus avenae* combined with green manure compost (GMC) on corky root disease in experiment 1. Nematode inoculation rate 3 nematodes ml⁻¹ substrate. Bars with different superscripts are significantly different at $p \leq 0.05$.

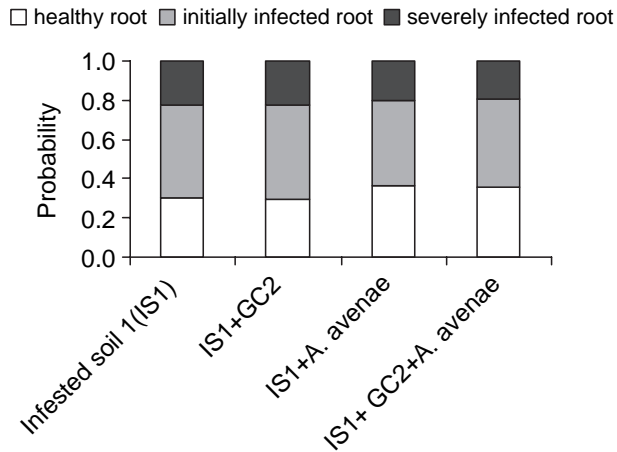


Figure 2. Effect of *Aphelenchus avenae* together with garden compost 2 (GC2) on corky root disease in experiment 2. Nematode inoculation rate 23 nematodes ml⁻¹ substrate.

lower when it was combined with nematodes. *Aphelenchus avenae* in this experiment exhibited the same trend as in experiment 1 and 2 in reducing disease in the infested soil in the absence of compost. The nematode significantly ($p < 0.0001$) reduced disease severity in the infested soil both at high (23 nematodes ml⁻¹ substrate) and low (3 nematodes ml⁻¹ substrate) inoculation rate (Figure 4).

Initial nematode population in soil, composts and soil-compost mixtures

Aphelenchus avenae was not found in the infested soil or in any of the composts while *Aphelenchoides* sp. was found in low numbers (0.2–0.8 nematodes ml⁻¹ substrate) in the infested soil and in all soil-compost mixtures. Bacterivorous and other (non-fungivorous) nematodes dominated in the infested soil and all composts. In GC2, a few individuals of the

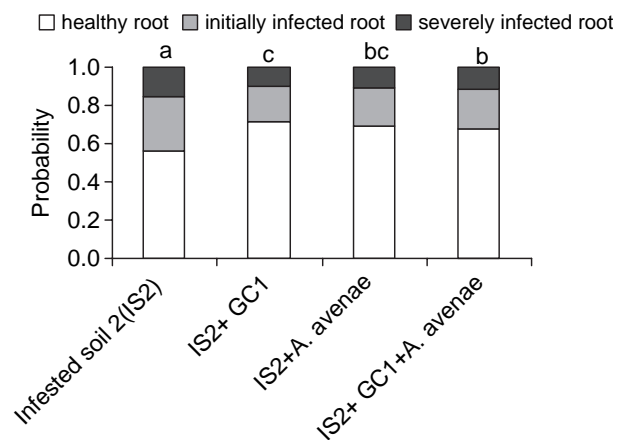


Figure 3. Effect of *Aphelenchus avenae* together with garden compost 1 (GC1) on corky root disease in experiment 3. Nematode inoculation rate 23 nematodes ml⁻¹ substrate. Bars with different superscripts are significantly different at $p \leq 0.05$.

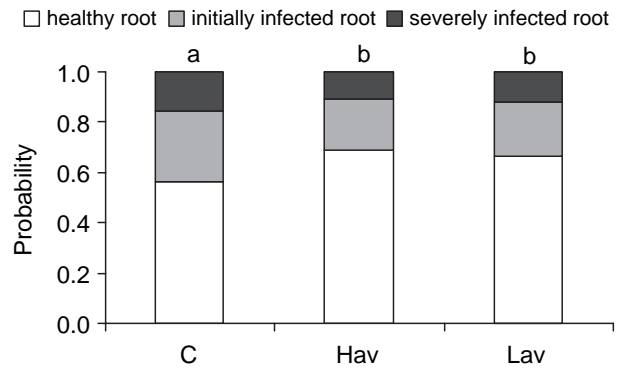


Figure 4. Effect of high and low inoculation rate of *Aphelenchus avenae* on corky root disease in the infested soil. C=Control without nematode addition, Hav=High inoculation rate of *A. avenae* (23 nematodes ml⁻¹ substrate), Lav=Low inoculation rate of *A. avenae* (3 nematodes ml⁻¹ substrate). Bars with different superscripts are significantly different at $p \leq 0.05$.

fungivorous *Ditylenchus* sp. were found (0.2 nematodes ml⁻¹ compost). The initial population of predatory nematodes and plant parasitic nematodes in different substrates was negligible.

Final population of nematodes

The final population density of inoculated nematodes for both *A. avenae* and *Aphelenchoides* spp. was low in all experiments. For *A. avenae*, the final population in compost-amended pots did not differ significantly from that in non-amended pots. The final number of *A. avenae* was not significantly higher in HMC-amended pots than in GC1-amended pots (Table I). In inoculated pots, the final population of *Aphelenchoides* spp. in GC2- and HMC-amended pots did not differ from that in non-amended pots. In GC1-amended pots, however, the final number of *Aphelenchoides* spp. was significantly ($p \leq 0.05$) lower than in non-amended pots (Table II). Increasing the inoculation rate from low to high did not increase the final number of nematodes for either *A. avenae* or *Aphelenchoides* spp.

Population development of *Aphelenchus avenae*

The initial number of *A. avenae* (23 nematodes ml⁻¹ substrate) decreased to 5 nematodes ml⁻¹ substrate five days after inoculation (Figure 5). Nematode numbers decreased further to 3 nematodes ml⁻¹ substrate after 10 days of inoculation, but afterwards increased significantly ($p \leq 0.05$) to 6 nematodes ml⁻¹ substrate after 15 days of inoculation. There was then a continual decrease in nematode numbers until the end of the experiment, when the population of *A. avenae* was 3 nematodes ml⁻¹ substrate.

Table I. Population density (ml⁻¹ substrate) of *Aphelenchus avenae* and other nematodes (mean ± SD) at the end of experiments 1–3. Values in the same column for individual experiments followed by different letters are significantly different at $p \leq 0.05$.

Treatment	Number of nematodes ml ⁻¹ substrate		
	<i>Aphelenchus avenae</i>	Other fungivorous nematodes ^a	Bacterivorous and other (non-fungivorous) nematodes
Experiment 1, $n = 3$			
IS1	0.0b ± 0.0	0.3a ± 0.2	62.3a ± 27.9
IS1+GMC	0.0b ± 0.0	0.2a ± 0.1	27.1a ± 16.5
IS1+ <i>A. avenae</i> (3 nematodes ml ⁻¹ substrate)	2.6a ± 1.2	0.0b ± 0.0	93.0a ± 77.7
IS1+GMC+ <i>A. avenae</i> (3 nematodes ml ⁻¹ substrate)	1.9a ± 1.3	0.0b ± 0.0	29.9a ± 43.9
Experiment 2, $n = 6$			
IS1	0.0b ± 0.0	0.0a ± 0.0	152.6a ± 47.6
IS1+GC2	0.0b ± 0.0	0.2a ± 0.2	80.2ab ± 39.7
IS1+ <i>A. avenae</i> (23 nematodes ml ⁻¹ substrate)	3.6a ± 1.0	0.0a ± 0.0	188.8a ± 81.5
IS1+GC2+ <i>A. avenae</i> (23 nematodes ml ⁻¹ substrate)	3.1a ± 1.5	0.0a ± 0.0	85.5ab ± 31.3
Experiment 3, $n = 8$			
IS2	0.0c ± 0.0	2.5a ± 1.2	77.1bc ± 22.2
IS2+HMC	0.0c ± 0.0	1.1ab ± 0.4	165.0a ± 41.0
IS2+GC1	0.0c ± 0.0	0.7ab ± 0.7	123.8ab ± 33.4
IS2+ <i>A. avenae</i> (23 nematodes ml ⁻¹ substrate)	2.7ab ± 1.3	0.4bc ± 0.4	67.3c ± 20.7
IS2+HMC+ <i>A. avenae</i> (23 nematodes ml ⁻¹ substrate)	3.6a ± 1.2	0.0c ± 0.0	151.5ab ± 23.7
IS2+GC1+ <i>A. avenae</i> (23 nematodes ml ⁻¹ substrate)	1.6ab ± 0.8	0.4bc ± 0.5	109.6b ± 25.4
IS2+ <i>A. avenae</i> (3 nematodes ml ⁻¹ substrate)	2.0ab ± 1.2	0.1bc ± 0.2	72.61bc ± 19.2

^a: Includes only *Aphelenchoides* spp. IS1: infested soil 1; IS2: infested soil 2; GMC: green manure compost; GC2: garden waste compost 2; HMC: horse manure compost; GC1: garden waste compost 1.

Effect on plant growth

Addition of any of the four composts to the infested soil did not significantly increase shoot and root dry weight and total fruit weight. *Aphelenchus avenae* alone increased shoot dry weight significantly ($p \leq 0.05$) in the infested soil in experiment 1 but not in other experiments. Application of *A. avenae* did not increase root dry weight in any of the experi-

ments. Fruit weight increased with the application of *A. avenae* to the infested soil alone only in experiment 3 (data not presented).

Discussion

Our results showed that *Aphelenchus avenae* decreased corky root disease severity (by around 10% compared with the control) when applied to infested

Table II. Population density (ml⁻¹ substrate) of *Aphelenchoides* spp. and other nematodes (mean ± SD) at the end of experiments 2, 3. Values in the same column for individual experiments followed by different letters are significantly different at $p \leq 0.05$. No other fungivorous nematodes were found. See Table I for explanation of treatments.

Treatment	Number of nematodes ml ⁻¹ substrate	
	<i>Aphelenchoides</i> spp.	Bacterivorous and other (non-fungivorous) nematodes
Experiment 2, $n = 6$		
IS1	0.0b ± 0.0	152.6a ± 47.6
IS1+GC2	0.2b ± 0.2	92.8ab ± 47.3
IS1+ <i>Aphelenchoides</i> spp. (33 nematodes ml ⁻¹ substrate)	0.5a ± 0.6	49.4bc ± 37.6
IS1+GC2+ <i>Aphelenchoides</i> spp. (33 nematodes ml ⁻¹ substrate)	0.5a ± 0.4	85.3abc ± 67.9
Experiment 3, $n = 8$		
IS2	2.5ab ± 1.2	77.1bc ± 22.2
IS2+HMC	1.1bc ± 0.4	164.9a ± 41.0
IS2+GC1	1.0bc ± 0.6	123.8ab ± 33.4
IS2+ <i>Aphelenchoides</i> spp. (33 nematodes ml ⁻¹ substrate)	3.2a ± 1.2	100.3bc ± 50.3
IS2+HMC+ <i>Aphelenchoides</i> spp. (33 nematodes ml ⁻¹ substrate)	2.5ab ± 1.0	138.2ab ± 43.9
IS2+GC1+ <i>Aphelenchoides</i> spp. (33 nematodes ml ⁻¹ substrate)	1.2bc ± 0.5	71.5bc ± 25.22
IS2+ <i>Aphelenchoides</i> spp. (3 nematodes ml ⁻¹ substrate)	2.7ab ± 1.8	104.1bc ± 52.6

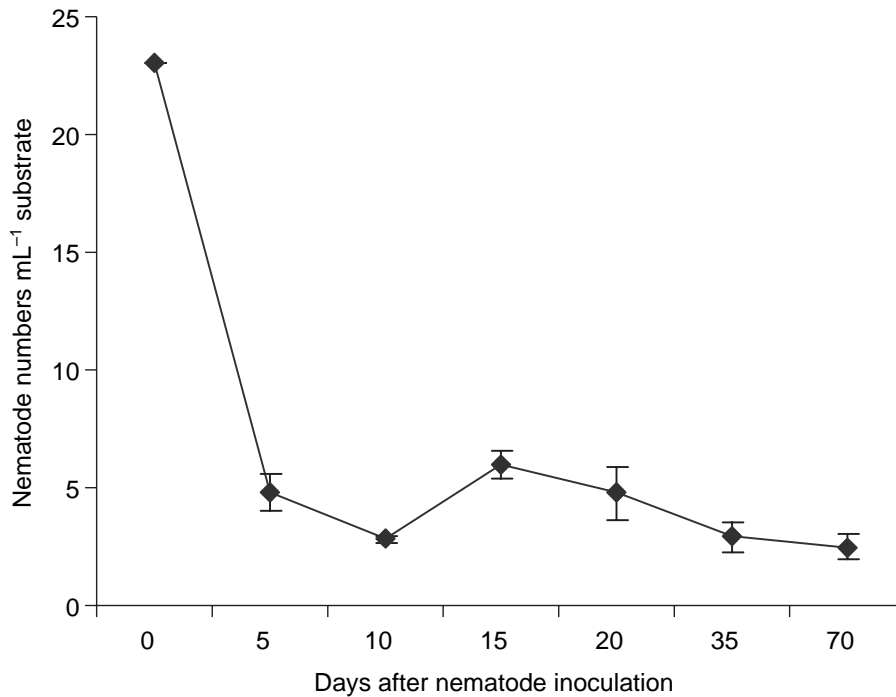


Figure 5. Population development of *Aphelenchus avenae* in the infested soil. Initial inoculation rate was 23 nematodes mL⁻¹ substrate. Nematode numbers (mean ± SD, $n=4$) were determined 5, 10, 15, 20, 35 and 70 days after inoculation.

soil without any compost amendment. However, *Aphelenchoides* spp. were unable to reduce the disease severity in any of our experiments. In reproduction testing of fungivorous nematodes on agar plates, *Aphelenchoides composticola* had been shown to reproduce well on culture of *Botrytis cinerea* Pers, whereas it did not reproduce on culture of dry-rot fungus *Merulius lachrymans* (Goodey & Hooper, 1962). This might be because *B. cinerea* was more palatable or at least more accessible to *A. composticola* than *M. lachrymans*.

In our study, it is possible that *P. lycopersici* was not sufficiently palatable to *Aphelenchoides* spp. nematodes to fulfil their nutrient requirements, whereas *A. avenae* could use this fungus successfully as food. A study by Ikonen (2001) indicates that *A. avenae* and *Aphelenchoides bicaudatus* Filipjev and Schuurman Stekhoven have different nutritional requirements. In our previous laboratory studies, both *A. avenae* and *Aphelenchoides* spp. showed a preference for *P. lycopersici* as a food source and multiplied very well in cultures of this fungus (Hasna et al., 2007). However, the *Aphelenchoides* spp. investigated might change their food preferences temporarily in the soil and select other soil fungi as their food source. The results from an *in vitro* study by Ruess et al. (2000) support this idea, as they observed a change in food source of an *Aphelenchoides* sp. when an alternative was available. According to them, the possible reason for this change in

feeding behaviour was to avoid undesirable toxic compounds in the food source.

The suppressive effect of *A. avenae* in the present study was lost when it was combined with green manure compost. The analysis of microbial activity in different composts showed that GMC had higher microbial activity than the other three composts (data not shown). This indicates that inoculated *A. avenae* had to compete for nutrients and space with other organisms in GMC-amended pots, so nematode activity could be hampered due to this competition.

The only compost that had a suppressive effect on *Pyrenochaeta* was GC1. This effect was lower when it was combined with fungivorous nematodes. It is not clear why the suppressive effect of GC1 was lower here. Fungivorous nematodes might use the beneficial fungi, which were present in GC1 and responsible for disease suppression, as a food source. However, further research is needed to establish whether beneficial fungi were involved in disease suppression and whether fungivorous nematodes preferred these fungi.

In all experiments, both *A. avenae* and *Aphelenchoides* spp. failed to maintain their initial population level. One factor causing reductions in nematode populations could have been food scarcity. Our greenhouse experiment continued for 10 weeks and the limited amount of substrate available was perhaps not sufficient to supply food for fungivorous nematodes for this long time period. However, the

involvement of some other factors such as natural enemies in the soil and/or abiotic factors cannot be excluded. In the population dynamic experiment, nematode numbers declined gradually from the initial level (23 nematodes ml⁻¹ substrate) to a low number (3 nematodes ml⁻¹ substrate) at the end of the experiment. Nematode population density in a certain soil is proportional to the amount of food available in that soil. A certain population density at which the food available in soil is just enough to maintain that population, is termed the equilibrium density (Seinhorst, 1966). The final number of *A. avenae* was 3 nematodes ml⁻¹ substrate, regardless of whether initial inoculation level was 3 nematodes ml⁻¹ substrate (experiment 1) or 23 nematodes ml⁻¹ substrate (experiments 2 and 3). Therefore, we can deduce that the equilibrium density of our experimental soil was 3 nematodes ml⁻¹ substrate. This low number of nematodes was probably sufficient to suppress *P. lycopersici*, since we observed disease reduction in our experiments. However, the increase in nematode population from 3 to 6 nematodes ml⁻¹ substrate after 10 days of inoculation could be related to the availability of mycelium after germination of microsclerotia of *P. lycopersici*, although the duration of *P. lycopersici* microsclerotia germination is not yet known.

In the present study, the disease severity in the infested soil decreased by approximately 28% in the second year. Loss of pathogenicity of *P. lycopersici* to tomato and melon is common (Clerjeau, 1974). However, the percentage improvement brought about by addition of *A. avenae* to the infested soil was almost the same (around 10%) in all of our experiments.

In our study, addition of composts did not increase root and shoot weight and total fruit weight. In general, disease suppression by *A. avenae* in the infested soil also did not increase root and shoot weight and total fruit weight. This could be because of the short experimental period and early harvesting.

In conclusion, our results suggest that the nematode *Aphelenchus avenae* can reduce corky root disease severity in tomato plants under greenhouse conditions. Among the composts tested, only a garden waste compost could suppress the disease. *Aphelenchus avenae* did not reinforce the suppressive effect of this compost.

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