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RESEARCH ARTICLE

Food attraction and population growth of fungivorous nematodes with different fungi

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Abstract

Food attraction of the fungivorous nematodes *Aphelenchus avenae* and *Aphelenchoides* spp. to seven fungal species (*Pyrenochaeta lycopersici*, *Botrytis cinerea*, *Rhizoctonia solani* strains AG 3 and AG 2-1, *Verticillium dahliae*, *Pochonia bulbillosa*, *Mortierella hyalina* and *Trichoderma harzianum*) was determined on agar plates by counting the number of test nematodes present on the mycelium of each fungus 24 h after inoculation. Population growth of *A. avenae* and *Aphelenchoides* spp. on five of the seven fungi included in the attraction test (*P. lycopersici*, *R. solani* strain AG 3, *V. dahliae*, *P. bulbillosa* and *T. harzianum*) was also determined on agar plates by counting nematode numbers every week during a 6-week period. *A. avenae* and *Aphelenchoides* spp. were attracted to all the fungi tested. *A. avenae* was preferentially attracted to *V. dahliae* ($P < 0.0001$), and *Aphelenchoides* spp. did not show any preference except for low attraction to *R. solani*. *A. avenae* and *Aphelenchoides* spp. reproduced on all fungal species tested. After 6 weeks of incubation, the highest number of nematodes was found on *P. lycopersici* and *P. bulbillosa*, while the lowest number occurred on *R. solani* for *A. avenae* and on *T. harzianum* for *Aphelenchoides* spp. The suitability of a fungus as a host was not clearly related to the attraction to that fungus.

Introduction

Nematodes are the most abundant multicellular organisms in the terrestrial ecosystem (Bongers & Bongers, 1998). The role of nematodes in soil communities is described according to the nature of food and mode of feeding as fungivorous, bacterivorous, omnivorous, predatory or plant parasitic (Nicholas, 1984; Freckman & Caswell, 1985). Fungivorous nematodes can act as biocontrol agents of various economically important fungal plant pathogens including *Fusarium*, *Pythium* and *Rhizoctonia* (Barnes *et al.*, 1981; Gupta, 1986; Ishibashi & Choi, 1991; Okada, 2006). In soil, the most common fungivorous nematodes include species belonging to the genera *Aphelenchus*, *Aphelenchoides*, *Ditylenchus* and *Tylenchus* (Freckman & Caswell, 1985).

Generally, fungivorous nematodes are polyphagous, feeding on a wide range of species of soil fungi including saprophytic, pathogenic, beneficial and mycorrhizal fungi (Freckman & Caswell, 1985; Giannakis & Sanders, 1989; Ruess & Dighton, 1996). Feeding on different fungi has differing impacts on soil ecology. When fungivorous nematodes have pathogenic fungi as their suitable hosts, disease suppression can be expected. In contrast, when antagonistic fungi (e.g. *Trichoderma*, *Gliocladium*) are preferred hosts, then the beneficial effect of these antagonists is reduced. However, fungivorous nematodes (Ruess & Dighton, 1996) and other mycophagous soil fauna, especially the Collembola (Warnock *et al.*, 1982), can negatively affect soil 'non-target' microorganisms such as mycorrhizal fungi by hyphal grazing. Thus, selective grazing by fungivorous nematodes has a significant impact on soil ecology, and

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research on food attraction is aimed at developing biocontrol strategies using fungivorous nematodes. In previous studies about the feeding behaviour of fungivorous nematodes (Ikonen, 2001; Okada & Kadota, 2003; Okada *et al.*, 2005), the focus has been on how they multiply with different fungi as food sources. Less information is available about the food selection of fungivorous nematodes when they are offered more than one fungus at a time. This information is important when fungivorous nematodes are added to the soil to control certain pathogenic fungi. In the soil environment, more than one fungus can be available to the fungivorous nematode and then its food attraction can play a decisive role for its potential effect. At the same time, it is important to know whether an attractive fungus is suitable for reproduction of the fungivorous nematode. Therefore, in the present study, we carried out three experiments in the laboratory. In the first experiment, we investigated the food attraction of the fungivorous nematodes *Aphelenchus avenae* Bastian and *Aphelenchoides* spp. to seven fungal species, including plant pathogenic and saprophytic fungi, by offering the fungi as food sources at the same time. Our hypothesis was that food attraction of the fungivorous nematodes to different ecological groups of fungi would vary. In a second experiment, the attraction of these fungivorous nematodes to three plant pathogenic fungi was compared with their attraction to one antagonistic fungus, *Trichoderma harzianum*. Our hypothesis was that the pathogenic fungi would be more attractive than the beneficial fungus. In the third experiment, we investigated the population development of *A. avenae* and *Aphelenchoides* spp. on five fungi during a 6-week period, where our hypothesis was that population development of fungivorous nematodes would be higher on the fungus that was most attractive to nematodes as a food source.

Materials and methods

Fungi

The experiments were carried out with seven different fungi: four plant pathogenic fungi (*Botrytis cinerea* Pers., *Pyrenochaeta lycopersici* R.W. Schneider & Gerlach, *Rhizoctonia solani* J.G. Kühn and *V. dahliae* Kleb.), two saprophytic fungi (*Mortierella hyalina* (Harz) W. Gams and *Pochonia bulbillosa* (W. Gams & Malla) Zare & W. Gams) and one antagonistic/saprophytic fungus (*T. harzianum* Rifai). Fungi from two ecological groups were chosen for better understanding of the role of fungivorous nematodes in the soil. *M. hyalina* and *T. harzianum* were isolated from a compost coded 'm' (wood chips 88%, manure 2.5% and clay 10%) originating from the Netherlands (Termorshuizen *et al.*, 2006), *B. cinerea* was isolated from strawberry (*Fragaria vesca* L.) in Sweden and *P. lycopersici* was isolated from tomato (*Lycopersicon esculentum* M.) in Sweden. Two strains of *R. solani* were used: AG 3 isolate UNI, obtained from potato (*Solanum tuberosum* L.) in the UK, and AG 2-1 isolate 21R21, obtained from cauliflower (*Brassica oleracea* L. var. *botrytis*) (kindly provided by Dr van der Gaag, Wageningen University and Research Centre, the Netherlands). *R. solani* AG 3 was used in the attraction test with seven different fungi and in the population growth test, while *R. solani* AG 2-1 was used in the attraction test with an antagonistic fungus versus three plant pathogenic fungi. *V. dahliae* was isolated from aubergine (*Solanum melongena* L.) in Greece. *P. bulbillosa* was isolated from coniferous forest soil in Sweden and was kindly provided by Dr Björn Sohlenius, the Swedish National Museum of Natural History.

Nematodes

Aphelenchus avenae was isolated from a potato field soil in Västergötland, Sweden (approximately 58°10′N, 13°34′E). *Aphelenchoides* spp. were isolated from a compost coded 'm' (the compost described above). Among *Aphelenchoides* spp., two species have been distinguished on the basis of morphological characteristics, but we have not further identified them. In our experiments, a proportionally similar mixture of two *Aphelenchoides* species isolated from the composts was used and is referred to here as *Aphelenchoides* spp.

The nematodes were propagated in cultures of *P. bulbillosa* grown in malt extract bacto agar (Difco Lab, Detroit, Michigan, USA). The initial cultivation of nematodes was successfully accomplished by picking out 5–10 specimens under a stereo microscope, surface sterilising them (0.5% chlorhexidine for 20 min and repeated rinsing in sterile water) and placing 50 μL of sterile water containing sterilised nematodes on fungal mycelium on agar plates (5 cm diameter). After 4 weeks, nematodes covered the whole plates. To obtain nematode inoculum for the experiments, two agar pieces (1.5 cm diameter) from a nematode propagation culture were cut out and placed in two opposing holes on the margin of a monoxenic culture of *P. bulbillosa* on agar plates (9 cm diameter). The plates were sealed and placed in the dark at 22°C for 4 weeks for nematode propagation, after which the nematodes were extracted from finely chopped agar by the Baermann funnel method (Southey, 1986).

Attraction tests

Attraction test to seven different fungi

The food attraction of *A. avenae* and *Aphelenchoides* spp. to seven different fungi was tested on 1% water agar plates (9 cm diameter). Four tests (I–IV) were performed with *A. avenae* at numbers of 600, 700, 1500 and 3000

individuals per plate with four, four, six and five replicates, respectively. Six tests (I–VI) were performed with *Aphelenchoides* spp. at numbers of 250, 600, 600, 700, 1200 and 2000 individuals per plate with three, four, five, three, four and four replicates, respectively. Initial number of nematodes and replicates in different tests were different because of the availability of nematodes. All fungi were grown on potato dextrose agar (PDA) (Merck Lab, Darmstadt, Germany). A mycelium-containing agar disc (0.5 cm diameter) from each of seven fungal cultures (10-day old) was taken and the seven agar discs were placed on a water agar plate in a ring 2 cm away from the central hole where nematode suspension in 100 μL of sterile water was placed. A PDA disc of the same diameter without fungi was also placed in the ring as a control. Before arranging the fungal discs, holes were made in the agar to keep nematodes and fungi on the same level. The nematode suspension and fungal discs were placed simultaneously on the agar plate, and all tests were conducted according to the same experimental procedure. The plates were incubated at 22°C in the dark for 24 h. Afterwards, one agar plug (1.0 cm diameter) from each fungal mat and from the control were extracted separately by the Baermann funnel method for 24 h. The remaining agar on each plate was extracted by the same method.

Attraction tests with an antagonistic fungus versus plant pathogenic fungi

Nematode attraction to *T. harzianum* was compared separately with that to three plant pathogenic fungi (*P. lycopersici*, *R. solani* AG 2-1 and *V. dahliae*) on 1% water agar plates (9 cm diameter). All fungi were grown on PDA. Two discs of one pathogen were matched against two discs of *T. harzianum* and two discs of agar without any fungi (control) at a time. The size of all discs was 0.5 cm (diameter). In the centre of the agar plates, the *A. avenae* suspension in 100 μL of sterile water was placed in a hole with *P. lycopersici*, *R. solani* or *V. dahliae* (2000, 3500 and 2000 individuals per plate, respectively) and the *Aphelenchoides* spp. suspension was placed in a hole with *P. lycopersici*, *R. solani* or *V. dahliae* (4000, 4000 and 3500 individuals per plate, respectively). Five replicates for each fungus–nematode combination were conducted and extracted as above.

Population growth test

Population growth tests of *A. avenae* and *Aphelenchoides* spp. were conducted with five fungal species (*V. dahliae*, *R. solani* AG 3, *P. lycopersici*, *T. harzianum* and *P. bulbillosa*) grown on PDA. A single plug (0.5 cm) of each fungus was placed in the centre of new agar plates (9 cm diameter). When the fungal colonies covered the plates, 30 nematodes of *A. avenae* or *Aphelenchoides* spp. (juveniles and adults) suspended in 50 μL of sterile water were inoculated in two opposite holes near the edge of each plate. In total, 60 plates were prepared for each fungus (30 for *A. avenae* and 30 for *Aphelenchoides* spp.). The plates were incubated at 22°C in the dark. The measurement of nematode population density started 1 week after inoculation and was carried out every week during a 6-week period. Five plates of each fungus and nematode were destructively sampled and extracted separately every week by the Baermann funnel method.

Statistical analysis

Nematode numbers in the attraction test were analysed using 'proc mixed' in SAS, version 8.2 (SAS Institute Inc., Cary, NC, USA), with initial nematode numbers and fungal species as fixed classification variables and extracted nematode numbers and plates as random classification variables. Initial nematode numbers were included as continuous covariates in the model. Post hoc comparisons were tested with least squares means. Nematode numbers in population growth test were analysed using ANOVA in SAS (version 8.2; SAS Institute Inc.) to determine significant differences between fungi within each week and significant differences between weeks within each fungus. The Bonferroni t test at $\alpha = 0.00037$ was used to calculate least significant difference (LSD) for pairwise comparisons. All data were log-transformed prior to analysis to make the variance homogeneous.

Results

Attraction test to seven different fungi

After 24 h of nematode inoculation, *A. avenae* and *Aphelenchoides* spp. migrated to all fungal areas and the control area (without fungus) in all tests. However, the number of nematodes extracted from the fungal areas was always significantly higher than that from the control area (Tables 1 and 2). For *A. avenae*, when all tests were pooled together, the results showed that the number of these nematodes attracted to *V. dahliae* was overall significantly ($P < 0.0001$) higher than for the other fungi tested (Table 1). However, this result varied for the individual tests. There was no difference between the fungi in numbers of nematode attracted in test I. In test II, the numbers of nematode attracted to *V. dahliae* was significantly higher ($P < 0.0001$) than that attracted to other fungi except *P. bulbillosa* and *T. harzianum*. In test III, the number of *A. avenae* attracted to *V. dahliae* was not

Table 1 Food attraction of *Aphelenchus avenae* to seven different fungi[a]

Test	Initial Number of Nematodes per Plate	Mean Number of Nematodes[b] (Extracted from One Agar Plug of 1.0 cm Diameter)								Probability Value
		Bc	Mh	Pb	Pl	Rs	Vd	Th	Control (Without Fungus)	
I, $n = 4$	600	68.5 b	42.0 b	44.0 b	52.8 b	64.0 b	64.3 b	nt	4.5 a	<0.0001
II, $n = 4$	700	34.5 bc	32.0 bc	40.0 cd	17.8 b	31.8 bc	92.5 d	52.8cd	0.0 a	<0.0001
III, $n = 6$	1500	83.0 b	138.3 bc	112.7 bc	194.0 bc	128.5 bc	543.7 c	nt	7.8 a	<0.0001
IV, $n = 5$	3000	226.2 bc	123.0 b	256.0 cd	385.2 de	133.8 bc	1444.4 e	nt	3.4 a	<0.0001
All tests (I–IV), $n = 19$		103.0 b	83.8 b	113.2 b	162.4 b	89.5 b	536.2 c	—	3.9 a	<0.0001

—, not included in statistical analysis; Bc, *Botrytis cinerea*; Mh, *Mortierella hyalina*; nt, not tested; Pb, *Pochonia bulbillosa*; Pl, *Pyrenochaeta lycopersici*; Rs, *Rhizoctonia solani* AG 3; Th, *Trichoderma harzianum*; Vd, *Verticillium dahliae*.
[a]Nematodes were extracted 24 h after inoculation. For each test, values followed by different letters are significantly different ($P < 0.05$).
[b]Includes adults and juveniles.

significantly different from that attracted to any other fungus except *B. cinerea* ($P < 0.0001$). In test IV, *V. dahliae* attracted significantly higher ($P < 0.0001$) number of nematodes than other fungi except *P. lycopersici*.

For *Aphelenchoides* spp., when all tests were pooled together, the results showed that *R. solani* AG 3 attracted significantly ($P < 0.0001$) lower number of nematodes overall than the other fungi tested (Table 2). When the tests were analysed separately, the number of *Aphelenchoides* spp. attracted to *R. solani* AG 3 was significantly lower than that attracted to *B. cinerea* and *P. bulbillosa* in test I ($P = 0.001$), to *B. cinerea* in test II ($P = 0.0001$), to all fungi in test IV ($P < 0.0001$), to *V. dahliae* and *T. harzianum* in test V ($P < 0.0001$) and to *V. dahliae* and *M. hyalina* in test VI ($P < 0.0001$).

Attraction test with an antagonistic fungus versus plant pathogenic fungi

The numbers of *A. avenae* and *Aphelenchoides* spp. attracted to *T. harzianum* and the three plant pathogenic fungi *P. lycopersici*, *R. solani* AG 2-1 and *V. dahliae* were significantly higher than those present in the control area (without fungus) (Table 3). *A. avenae* was significantly more attracted to *T. harzianum* than to *R. solani* AG 2-1, while *Aphelenchoides* spp. did not show any difference in attraction between *T. harzianum* and *R. solani* AG 2-1. There was no difference in numbers of *A. avenae* and *Aphelenchoides* spp. attracted to *T. harzianum*, *P. lycopersici* and *V. dahliae*.

Population growth tests

The LSD test showed that after the 6-week period, the significantly highest numbers of *A. avenae* and *Aphelenchoides* spp. were found on *P. lycopersici* and *P. bulbillosa*, while the lowest number was found on *R. solani* for *A. avenae* and on *T. harzianum* for *Aphelenchoides* spp. (Figs 1 and 2). Nematode numbers increased significantly on *P. bulbillosa* and *P. lycopersici* until weeks 4 and 3, respectively, and remained approximately constant in the following weeks. Numbers of *A. avenae* and *Aphelenchoides* spp. on *R. solani* and *V. dahliae* increased significantly from week 1 to 2 and remained approximately constant in the following weeks. Numbers of *A. avenae* and *Aphelenchoides* on *T. harzianum* increased initially, but after week 3, the numbers started to decrease.

Table 2 Food attraction of *Aphelenchoides* spp. to seven different fungi[a]

Test	Initial Number of Nematodes per Plate	Mean Number of Nematodes[b] (Extracted from One Agar Plug of 1.0 cm Diameter)								Probability Value
		Bc	Mh	Pb	Pl	Rs	Vd	Th	Control (Without Fungus)	
I, $n = 3$	250	48.0 c	33.3 bc	39.0 c	25.0 bc	15.7 b	24.7 bc	nt	0.0 a	0.0010
II, $n = 4$	600	146.0 c	97.0 bc	64.5 b	103.0 bc	46.2 b	43.0 b	nt	2.2 a	0.0001
III, $n = 5$	600	57.0 b	89.0 b	54.6 b	31.4 b	48.0 b	66.0 b	nt	9.8 a	0.0050
IV, $n = 3$	700	102.3 cd	50.3 c	136.7 d	158.7 d	6.0 b	115.0 d	nt	2.0 a	<0.0001
V, $n = 4$	1200	100.2 bcd	124.7 cd	30.2 ab	37.7 ab	61.5 bc	178.5 d	223.5 d	24.2 a	<0.0001
VI, $n = 4$	2000	125.5 b	391.3 d	278.5 cd	250.5 cd	147.0 bc	497.0 d	nt	39.5 a	<0.0001
All tests (I–VI), $n = 23$		96.5 c	130.9 c	100.6 c	101.2 c	54.1b	154.0 c	—	12.9 a	<0.0001

—, not included in statistical analysis; Bc, *Botrytis cinerea*; Mh, *Mortierella hyalina*; nt, not tested; Pb, *Pochonia bulbillosa*; Pl, *Pyrenochaeta lycopersici*; Rs, *Rhizoctonia solani* AG 3; Th, *Trichoderma harzianum*; Vd, *Verticillium dahliae*.
[a]Nematodes were extracted 24 h after inoculation. For each test, values followed by different letters are significantly different ($P < 0.05$).
[b]Includes adults and juveniles.

Table 3 Food attraction test with *Trichoderma harzianum* versus the plant pathogenic fungi *Pyrenochaeta lycopersici*, *Rhizoctonia solani* AG 2-1 and *Verticillium dahliae* to *Aphelenchus avenae* and *Aphelenchoides* spp.[a]

Test	Nematodes	Initial Number of Nematodes per Plate	Mean Number of Nematodes[b] (Extracted from Two Agar Plugs of 1.5 cm Diameter)			Probability Value
			T. harzianum	Plant Pathogenic Fungi	Control (Without Fungus)	
T. harzianum versus	*A. avenae*	2000	525.6 b	606.2 b	8.4 a	<0.0001
P. lycopersici, n = 5	*Aphelenchoides* spp.	4000	1011.2 b	1681.8 b	304.0 a	0.0004
T. harzianum versus	*A. avenae*	3500	2525.6 c	402.4 b	10.8 a	<0.0001
R. solani, n = 5	*Aphelenchoides* spp.	4000	1822.6 b	1155.7 b	77.2 a	0.0002
T. harzianum versus	*A. avenae*	2000	621.4 b	1155.6 b	19.2 a	0.0002
V. dahliae, n = 5	*Aphelenchoides* spp.	3500	1352.2 b	1572.0 b	20.2 a	0.0001

[a]Nematodes were extracted 24 h after inoculation. Values within rows followed by different letters are significantly different ($P < 0.05$).
[b]Includes adults and juveniles.

Discussion

In the present work, we studied the behaviour of fungivorous nematodes on the basis of their food attraction and population development on plant pathogenic and saprophytic fungi with the aim of developing better biocontrol strategies using fungivorous nematodes. From biocontrol point of view, the ideal situation would be for pathogenic fungi to be the most attractive and also the most suitable for reproduction of fungivorous nematodes. In our results, *A. avenae* was more attracted to *V. dahliae*, a pathogenic fungus, than to the other fungi tested. Anyhow, it should be kept in mind that we tested the mycelia of *V. dahliae*, while the fungus occurs in the soil in the form of microsclerotia, a highly resistant structure on which the nematode probably cannot feed until the microsclerotia germinate by the stimulation of host root exudates. In the population growth test, although nematode population on *V. dahliae* increased initially, nematode numbers were subsequently higher on *P. lycopersici* and *P. bulbillosa* than on *V. dahliae*. Thus, the results from our attraction and population growth tests indicate that the attraction intensity of a fungus as food is not always related to its suitability as a host of fungivorous nematodes on PDA medium. Similar results were obtained for fungivorous nematodes by Townshend (1964) and Ruess *et al.* (2000). However, our results indicate that the test nematodes, *A. avenae* and *Aphelenchoides* spp., may have potential as biocontrol agents against the two plant pathogens *P. lycopersici* and *V. dahliae*.

In the attraction tests, nematodes were extracted 24 h after inoculation. This period was more than enough for the nematodes to migrate to their food sources. Townshend (1964) reported that most nematodes responded

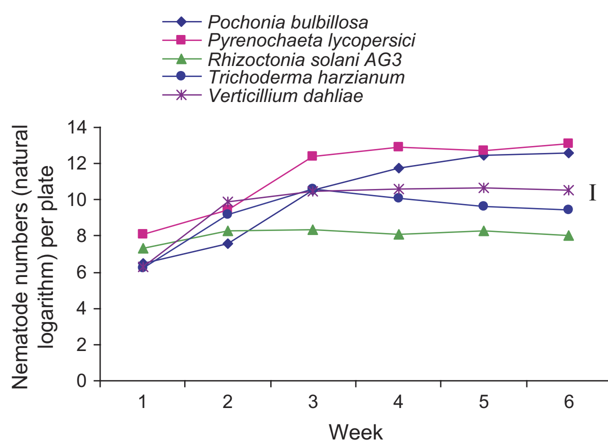

Figure 1 Population growth of *Aphelenchus avenae* on five different fungi. Nematode numbers extracted 1, 2, 3, 4, 5 and 6 weeks after inoculation in the fungal culture at an initial number of 30 individuals per plate. The vertical bar to the right in the figure represents least significant difference value (d.f. = 120, n = 5, α = 0.00037).

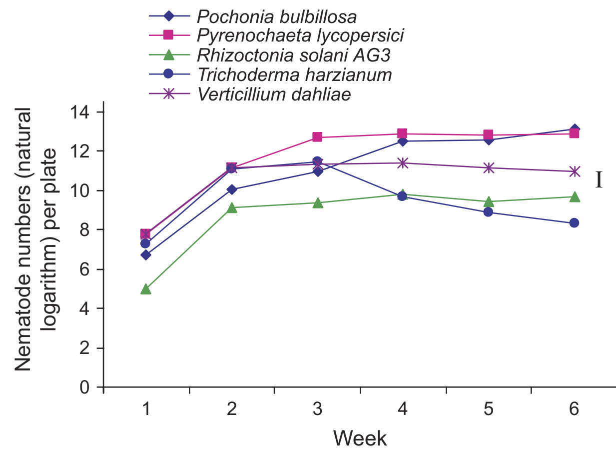

Figure 2 Population growth of *Aphelenchoides* spp. on five different fungi. Nematode numbers extracted 1, 2, 3, 4, 5 and 6 weeks after inoculation in the fungal culture at an initial number of 30 individuals per plate. The vertical bar to the right in the figure represents least significant difference value (d.f. = 120, n = 5, α = 0.00037).

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immediately to the fungal colonies in a food attraction study of *A. avenae* and *Bursaphelenchus fungivorous* to 59 fungus species on agar plates. In a study of interactions between nematophagous fungi and plant parasitic nematodes on agar plates, two plant parasitic nematodes, *Aphelenchoides fragariae* and *Ditylenchus destructor*, also known as fungus feeder (Faulkner & Darling, 1961; Goodey, 1963), were attracted to all tested fungi within 24 h after nematode introduction (Jansson & Nordbring-Hertz, 1980). The actual substances responsible for attraction of nematodes to fungi are unknown. In a study of nematode attraction to living mycelium of nematophagous fungi, conducted on agar plates, Jansson & Nordbring-Hertz (1979) suggested that volatile substances or diffusing compounds were responsible for attraction of nematodes to fungi.

During the 6-week growth period, population development of *A. avenae* and *Aphelenchoides* spp. was considerably different on the fungi tested. Fungivorous nematodes can use many fungal species as their food source, but some fungi support nematode populations better than others (Ikonen, 2001). To use fungus as food, nematodes need to puncture the fungal cell wall, which differs in chemical composition as well as thickness from fungus to fungus (Killham, 1994). A thin cell wall is easy to penetrate, and thus, the fungus possessing this type of cell wall is probably more suitable as a host. The chemical composition of the fungus has also been suggested as a factor that can influence the suitability of the fungus as a host (Ikonen, 2001). In the present study, the same PDA medium was used throughout to provide a homogeneous growth substrate for all fungi, as the culture medium can influence the mycelial growth of a fungus (Kim *et al.*, 2005), which in turn can affect nematode population growth. In a study by Okada *et al.* (2005), it was found that the suitability of a fungus for reproduction of fungal-feeding nematodes differed between two culture media (PDA and soil medium).

In our study, the nematodes were not highly attracted to *R. solani*, and this fungus was not a good host for either of the nematode genera, although Okada & Kadota (2003) found *R. solani* to be a good host for *A. avenae*. This variability is reported by other studies suggesting that strains of *R. solani* originating from different plant species vary greatly in their capacity to support population growth of *A. avenae* (Caubel *et al.*, 1981). Ikonen (2001) also reported different population growth of *A. avenae* and *Aphelenchoides bicaudatus* on two strains of *Penicillium restrictum*.

In the population growth test, nematode numbers on *P. bulbillosa* (saprophytic fungus) and *P. lycopersici* (plant pathogenic fungus) continued to increase until weeks 4 and 3, respectively. Afterwards, nematode numbers remained approximately constant until week 6 in both cases, probably because of shortage of food within the agar plates. Plant pathogenic fungi such as *Pyrenochaeta* sp., *R. solani* and *Verticillium albo-atrum* were found to be good hosts for *A. avenae* in a population development test of the nematode on 18 soil fungi in agar plates (Mankau & Mankau, 1963). However, suitability of a saprophytic fungus, *P. bulbillosa*, for population growth of fungivorous nematodes found in the present study is contradictory to the findings of Ruess & Dighton (1996) who found meagre populations of *A. avenae* and *Aphelenchoides saprophilus* on saprophytic fungi. In our study, nematodes were initially grown on *P. bulbillosa* for mass culture, and this might have influenced the increased number of nematodes on this fungus. Nematode numbers on *T. harzianum* started to decrease after week 3, a trend not found in the other fungi. Several reports showed that *Trichoderma* spp. are able to suppress plant parasitic nematodes (Windham *et al.*, 1993; Rao *et al.*, 1998; Sharon *et al.*, 2001). It was reported that a trypsin-like protease, isolated from culture filtrates of *T. harzianum* CECT 2413, reduced egg hatching in *Meloidogyne incognita*, a root knot nematode (Suarez *et al.*, 2004). However, large quantities of secondary metabolites of antagonistic fungi are not produced during normal vegetative growth but occur in circumstances where mycelial growth has ceased (Faull, 1988). It is possible that the fungal colony was favourable for nematodes to multiply at the beginning of the test but that the nematode population then started to decrease because of the production of toxic compounds as a defence mechanism by this antagonistic fungus.

Population growth rates of nematodes are affected by temperature (Anderson & Coleman, 1982; Vancoppenolle *et al.*, 1999; Okada & Ferris, 2001). The optimum temperature for population development of *A. avenae* is about 28°C (Pillai & Taylor, 1967; Mendis & Evans, 1983; Okada & Ferris, 2001), while individual species of the genus *Aphelenchoides* have different optimum temperature for reproduction, such as 20–24°C for *Aphelenchoides hamatus* (Rössner & Nagel, 1984), 28°C for *Aphelenchoides rutgersi* (Moens *et al.*, 1996) and 23°C for *Aphelenchoides composticola* (Okada & Ferris, 2001). In the present population growth test, the temperature was the same (22°C) for *A. avenae* and *Aphelenchoides* spp. As we did not identify the two species of *Aphelenchoides* that were used in the present study, the optimal temperature for reproduction is unknown and so we cannot say whether *A. avenae* or *Aphelenchoides* spp. was the most favourable by the chosen temperature.

In conclusion, our results suggest that there was no clear relationship between food attraction and population development of the fungivorous nematodes. However,

these two characteristics may influence the population of fungivorous nematodes in the soil. The food attraction characteristic helps nematodes to disperse all through the soil and thereafter they can multiply on their suitable hosts. The present study was observed on PDA medium in a controlled environment, but nematodes in the soil condition are influenced by a number of factors. Thus, many possible interactions are omitted in a laboratory test. Therefore, further examination of the soil environment is needed for better understanding of relationships between the organisms investigated here.

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