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Interactions between root-feeding nematodes depend on plant species identity

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ABSTRACT

Root-feeding nematodes play an important role in structuring the composition of natural plant communities. Little is known about the role of intra- and interspecific interactions in determining the abundance of root-feeding nematodes in natural ecosystems. We examined interactions between two ectoparasitic root-feeding nematodes on two plant species: a good host plant for both nematode species and a good host for only one of the nematodes. We tested the hypothesis that root herbivore competitiveness depends on host suitability and related the experimental results to field data. In a greenhouse, we added different densities of the nematodes *Tylenchorhynchus microphasmis* and *Tylenchorhynchus ventralis* to *Ammophila arenaria* (the good host for both) and *Carex arenaria* (a good host for *T. microphasmis* only). Addition of *T. ventralis* did not significantly affect multiplication of *T. microphasmis* on both plant species. In contrast, on *A. arenaria*, *T. ventralis* experienced interspecific competition. However, on *C. arenaria*, *T. microphasmis* facilitated multiplication of *T. ventralis*. To explain this effect, we studied systemic plant-mediated effects in a split-root experiment. Nematode addition to one root compartment did not significantly influence nematode multiplication in the other root compartment, irrespective of nematode species identity. Therefore, the observed nematode interactions were not related to induced changes in the roots. In a two-choice experiment we tested whether host suitability was related to root attractiveness. Both nematode species were attracted to seedlings of *A. arenaria*, but not to *C. arenaria*. The low multiplication of *T. ventralis* on *C. arenaria* could be related to poor attraction to the roots. However, the poor attraction of *T. microphasmis* cannot be related to poor host suitability. Adding *T. ventralis* reduced shoot biomass of *A. arenaria* more than *T. microphasmis* did, whereas for *C. arenaria* the effect was the reverse. The interaction of the two nematodes on *A. arenaria* and *C. arenaria* shoot biomass was insignificant. However, the effect on root biomass of *A. arenaria* was interactive; adding *T. ventralis* to plants with high inoculation densities of *T. microphasmis* further decreased root biomass. Adding *T. microphasmis* further decreased root biomass of plants inoculated with low levels of *T. ventralis*. Depending on host plant identity, interactions between root-feeding nematodes may lead to competition or facilitation. Our results suggest that facilitation by *T. microphasmis* contributes to persistence of *T. ventralis* on *C. arenaria*. Thus, the population dynamics of root-feeding nematodes is influenced both by host plant identity and the presence of other root-feeding nematodes.
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1. Introduction

Root-feeding nematodes and larval stages of insects are the main herbivores that use plant roots as a resource (Brown and Gange, 1990). In many soils the diversity of nematodes is high (Bongers and Bongers, 1998) and interactions, ranging from competitive to neutral to facilitative, among different nematode species will occur (Eisenback, 1993). The majority of nematode interaction studies have been carried out with single host plant species. Recently, it was shown that root-feeding nematodes may interact indirectly when feeding on different plant species within the same plant community (De Deyn et al., 2007). Here, we show how two ectoparasitic root-feeding nematodes may interact when feeding on the same plant, while using plant species of good and poor host quality. We tested the hypothesis that poor host plant quality reduces nematode competitiveness.

The different feeding strategies that are present among root-feeding nematodes enable exploitation of different resources from epidermal cells by ectoparasites, deeper cortical layers by migratory and semi-endoparasites, and phloem by sedentary endoparasites

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(Yeates et al., 1993). It is generally supposed that nematodes with comparable feeding habits influence each other more than nematodes with dissimilar feeding habits, although there are many exceptions to that rule (reviewed in Eisenback, 1993). Most studies on interactions between root-feeding nematodes have been performed with cultivated plants. As in natural soils interactions between nematodes and their host plants have had a longer time to co-evolve (Brinkman et al., 2005), studying these interactions in nature may help to explain how competition contributes to structuring nematode communities in relatively undisturbed systems.

Most studies on nematode interactions have focused on the influence of the interaction on nematode multiplication (Eisenback, 1993), whereas relatively few attempts have been made to unravel the underlying mechanisms (e.g. Lasserre et al., 1994; Umesh et al., 1994). Interactions between nematodes are density-dependent and act intra- as well as interspecifically. Root-feeding nematodes may directly compete by occupation or destruction of feeding sites (contest competition). Indirectly, they may influence each other by altering the physiology of their host plants (scramble competition, Eisenback, 1993). However, it is not known how nematode interactions may depend on resource availability or quality.

We used *Tylenchorhynchus microphasmis* and *Tylenchorhynchus ventralis*, two root-feeding nematodes that occur in coastal dunes (De Rooij-van der Goes et al., 1995), to investigate competitive and facilitative interactions between two belowground herbivores with ectoparasitic feeding strategies (Yeates et al., 1993). *Tylenchorhynchus* spp. puncture root hairs and epidermal cells, feeding upon the cytoplasm, and can be regarded as feeding-generalists. The stylet length of adult *T. microphasmis* and of adult *T. ventralis* is 21–28 μm and 16–19 μm, respectively (Brzeski, 1998), so that, with an estimated epidermal cell diameter of $(1.0–1.5)10^2$ μm (Purer, 1942; De Rooij-van der Goes, 1995), they may use the same cell layers for feeding (Yeates, 1986). However, it has also been reported that *T. ventralis* may enter the root centrally to a depth of two cells and laterally over four cells, showing semi-endoparasitic behaviour (De Rooij-van der Goes, 1995).

In greenhouse experiments, *T. ventralis* reproduces on *Ammophila arenaria* (marram grass) (De Rooij-van der Goes, 1995; Brinkman et al., 2004), whereas densities remain relatively low on *Carex arenaria* (sand sedge) (W.H. van der Putten, unpublished results). In contrast, *T. microphasmis* reproduces well both on *A. arenaria* and on *C. arenaria* (W.H. van der Putten, unpublished results). Based on these observed differences in host suitability, we assumed that when both nematode species co-occur in the root zone of *C. arenaria*, *T. ventralis* will be out competed by *T. microphasmis*. To unravel the mechanism of the interaction, we performed a split-root experiment to assess whether the effects would be of systemic nature. In addition, we carried out attraction experiments to determine a possible relationship between poor performance of *T. ventralis* on *C. arenaria* and host recognition.

2. Materials and methods

2.1. Field survey

Soil samples were taken on four dates (April, June, August and October) at two coastal sand dune locations (Autostrand and Haringvlietdam) at Voorne, the Netherlands (51°52′N; 04°04′E). From each site and harvest date, four soil samples were collected ($n = 4$) from vigorous and degenerate *A. arenaria* and from *C. arenaria*. However, in April eight soil samples were collected from vigorous *A. arenaria* ($n = 8$). Roots were separated from the soil by sieving, after which the soil of each separate sample was homogenized. Nematodes from 250 ml soil samples (density of 1.4 kg/l soil) were extracted by Oostenbrink elutriation (Oostenbrink, 1960), followed by sieving on one 75 μm and three 45 μm mesh sieves that caught the greater portion of the nematodes (Verschoor and De Goede, 2000). The debris from the sieves was transferred to a double cotton wool filter (Hygia rapid, Hartmann AG, Heidenheim, Germany) on a sieve in a dish with a layer of tap water (Oostenbrink, 1960), after which the nematodes were allowed to migrate through the filter for 24 h (16–25 °C). Nematodes from all the roots of a sample were extracted by the funnel-spray method (Oostenbrink, 1960) for 24 h. The nematode samples were stored at 4 °C until counting, using an inverted light microscope (50–200x magnification).

2.2. Soil, plant material and inoculum for the experiments

Soil used in the competition experiment and in the split-root experiment was collected around *A. arenaria* stands at the Haringvlietdam location. The soil was sieved, homogenized and sterilized by gamma-radiation (≥25 kGray). This dosage has been shown to effectively eliminate all nematodes from dune soil (De Rooij-van der Goes, 1995). Seeds of *A. arenaria* and *C. arenaria* were collected from the same site, dried and stored at 4 °C until use. The seeds were germinated on glass beads for 3 weeks at 30 °C/20 °C and 25 °C/10 °C, respectively, with 16 h light/8 h dark, after which the seedlings were planted (competition and split-root experiment). Nematodes originated from the same Haringvlietdam site as the soil and the plant material. Pure cultures, not containing other root-feeding nematode species, of *T. microphasmis* and *T. ventralis* were established and maintained on *A. arenaria* (established from seeds) in 20 l pots filled with sterilized dune soil. The cultures were kept in a greenhouse with controlled climate conditions and they were watered and provided with Hoagland nutrient solution weekly (De Rooij-van der Goes, 1995). Extractions containing all post-hatching life stages of the nematodes were obtained from the cultures and used for the different experiments.

2.3. Competition experiment

The interaction between the nematodes was studied in a full factorial design. Four different densities of *T. ventralis* and of *T. microphasmis* were added to pots either containing *A. arenaria* or *C. arenaria* ($4 \times 4 \times 2$ treatments). Each treatment was replicated six times. At the end of the experiment, densities of both nematode species and plant biomass were determined in each pot.

Pots (1.5 l) were filled with 1448 g (10% gravimetric soil moisture) sterilized soil and either four *A. arenaria* or four *C. arenaria* seedlings were planted in each pot. The soil was covered with aluminium foil to decrease evaporation. The first week after planting, dead seedlings were replaced by new ones. The plants were grown in a greenhouse at 21 °C and provided with extra light to ensure a minimum photosynthetic photon fluence rate of 200 μmol photons $m^{-2} s^{-1}$ over the waveband 400–700 nm during 16 h per day. Three weeks after planting, nematodes were added to the plants in four different densities: 0, 50, 250 and 1500 nematodes per pot. The nematodes were added in holes 2 cm away from the roots and the holes were closed by flushing with tap water. Once per week, the soil moisture was adjusted to 10% by adding demineralised water. Once a week, nutrients were added as Hoagland solution (Hewitt, 1966) in an increasing dosage to meet enhanced plant demand. The first 3 weeks, 25 ml half strength Hoagland solution was added, weeks 4–6, 25 ml full strength, weeks 7 and 8, 50 ml full strength and weeks 9–12, 75 ml full strength Hoagland was added (Van der Putten et al., 1988).

Twelve weeks after the start of the experiment, the plants were harvested. The soil was washed from the roots and shoot, rhizome (*C. arenaria*), and roots were separated. The plant material was dried for 48 h at 70 °C and then weighed. Nematodes were extracted from the soil by decantation (Van der Stoel et al., 2002).

The soil with nematodes was washed in a bucket and tap water was added to achieve 4–5 l suspension. The suspension was stirred and after waiting for 5–10 s, the water and the suspended nematodes were decanted through one 75 μm and three 45 μm sieves. This procedure of adding water, stirring and decanting was carried out four times. Nematodes in the debris from the sieves were extracted and counted as described in the field survey (Section 2.1).

2.4. Split-root experiment

Seedlings of *A. arenaria* and *C. arenaria* were pre-cultured for 2 months in sterilized dune soil, after which the roots were washed, split and planted in two adjacent pots with sterilized soil. Each pot contained 1.47 kg sterilized soil with a gravimetric soil moisture of 10% and was covered with aluminium foil to reduce evaporation. One day after splitting the roots, no nematodes, or 350 individuals of either *T. ventralis* or *T. microphasmis* were added to each of the two adjacent pots. All possible addition combinations were made, resulting in six different treatments, with four replicates for *A. arenaria* and six replicates for *C. arenaria* (due to higher variability of *C. arenaria* seedling size). The plants were grown in a greenhouse at 20 °C and provided with extra light to ensure a minimum photosynthetic photon fluence rate of 200 μmol photons m^{-2} s^{-1} over the waveband 400–700 nm during 16 h per day. Three times per week, the soil moisture was adjusted to 10% with demineralised water, dividing the water equally over the two root compartments. Once a week, nutrients were added as half strength Hoagland solution (Hewitt, 1966), divided equally over the two root compartments. During the first 2 weeks of the experiment, each plant was provided with 6 ml nutrient solution, which in the following 4 weeks was increased to 12 ml. The plants were harvested 6 weeks after nematode addition, following the procedure of the competition experiment, with two exceptions. First, rhizome and roots of *C. arenaria* were not separated and second, nematodes from the roots were extracted exposing half of each root sample to the funnel-spray method (Oostenbrink, 1960).

2.5. Attraction experiment

Nematode attraction to the different plant species was determined on water agar plates, using an adaptation of a method developed by Lavallee and Rohde (1962). The water agar medium enables instant counting of the nematodes and repeated measurements on the same entity, in contrast to experiments performed in sand. A Petri dish was divided into four quadrants, of which two opposite quadrants contained plants. After inoculating the nematodes to the centre of the Petri dish, the migration of the nematodes to the two treated quadrants was determined. All possible combinations of *A. arenaria*, *C. arenaria* and no plant were used, resulting in six separate experimental choice situations.

Seeds were surface sterilized in a 1% sodium hypochlorite (NaOCl) solution for 30 min, then washed three times with sterilized tap water. The *A. arenaria* and *C. arenaria* seeds were germinated on sterile filter paper in Petri dishes at 25 °C/22 °C with 16 h light/8 h dark for 7 and 16 days, respectively. Seedlings with a shoot length of 5–10 mm were planted in 8.5 cm diameter Petri dishes containing 3 ml (*T. microphasmis*) or 6 ml (*T. ventralis*) 0.75% water agar. The seedlings were grown for 6 days at 22 °C with 16 h light/8 h dark.

Nematode suspensions were obtained from a greenhouse culture and contained a mixture of root-feeding and microbial feeding nematodes. Microbial feeding nematodes may affect nutrient availability to the plants. However, as they were present in all treatments, we considered their effect to be a background for the whole experiment. The nematode suspensions were sieved several times over a 75 μm sieve to decrease the fraction of microbial feeding nematodes from 0.6 to 0.1, facilitating easier quantification of the root-feeding nematodes. Equal parts of the nematode suspension and a 1.5% water agar solution were mixed. Suspension (0.90 μl) containing 61 ± 24 *T. microphasmis* or 46 ± 16 *T. ventralis*, was pipetted in an 8 mm diameter hole in the centre of the agar plates. Migration of *T. microphasmis* was determined 3, 16, 32, 47 and 63 h after inoculation and of *T. ventralis* after 4, 19, 34 and 49 h. The experiment with *T. ventralis* was terminated earlier, because this nematode species migrated away from the centre sooner after inoculation and the migrated fraction did not further increase. During counting, all Petri dishes were kept at 4 °C to minimize migration.

2.6. Data analysis

Nematode abundance on the different plants in the field survey did not meet requirements for ANOVA, so that the data were analyzed by a three-way Scheirer–Ray–Hare test, with plant species, location and time as factors. Scheirer–Ray–Hare is an extension to the Kruskal–Wallis test, performing ANOVA on ranked values (Sokal and Rohlf, 1995). Approximate *P*-values for the *H*-statistic (sums of squares of the ranks divided by the total mean square of the ranks) were obtained from Rohlf and Sokal (1981).

In the competition experiment, plant biomass and nematode multiplication were analyzed by a Generalized Linear Model (normal distribution, identity link function). Plant species was used as categorical factor and added nematode densities (*T. ventralis* and *T. microphasmis*), including polynomials to the second degree (accounting for curvilinear response), as continuous factors. The set-up of the analysis was full factorial, but non-significant factors were left out from the model and only significant factors are presented (Crawley, 2005). Nematode multiplication only was determined in those treatments where nematodes had been added, not in the control without nematode addition. Therefore, different subsets of the experiment were used to analyze multiplication of each nematode species. The nematode data were $\ln(x+1)$-transformed to achieve a homogeneous distribution of the variance of the residuals.

In the split-root experiment, the effect of plant species and of nematode addition on (square-root transformed) total root dry biomass was analyzed by two-way ANOVA. The effect of plant species and nematode addition on shoot dry biomass was analyzed by a two-way Scheirer–Ray–Hare test, because the data did not meet the requirements for ANOVA. Within each plant species and nematode addition treatment, differences in root biomass between the two compartments were analyzed by a two-way paired comparisons ANOVA, with 'nematode addition' as one and 'replicate number' as the other factor. Final numbers of nematodes were analyzed by two-way ANOVA with plant species as one and nematode addition (no nematodes, the same or the other nematode species) to the adjacent root compartment as the other factor. The numbers of nematodes were $\ln(x+1)$-transformed to obtain homogeneity of variances.

In the attraction experiment, the fraction of inoculated nematodes that migrated to the zones of interest was analyzed by repeated measures ANOVA with factors 'plant combination' and 'time'. At $t = 32$ (*T. microphasmis*) and $t = 34$ (*T. ventralis*), for each of the different treatments a *G*-test was used to determine if the nematode distribution differed from a random distribution ($p = q = 0.5$, two-tailed, $\alpha = 0.05$) (Sokal and Rohlf, 1995).

3. Results

3.1. Field survey

In the root zones of *A. arenaria* and *C. arenaria*, the genus *Tylenchorhynchus* consisted of three species. At both locations, the

Table 1
Field survey

Plant/location		April	June	August	October
T. ventralis					
A. arenaria (vig.)	A	2.1 ± 1.58	0.5 ± 0.47	0	0.7 ± 0.48
	H	2.1 ± 0.90	0	0	0
A. arenaria (deg.)	A	1.4 ± 1.43	0	1.4 ± 1.43	0
	H	2.0 ± 1.35	0	1.4 ± 1.43	0
C. arenaria	A	0	0	0	2.9 ± 1.65
	H	0	0	0	0
T. microphasmis/T. nanus					
A. arenaria (vig.)	A[a]	11.4 ± 6.72	0	0	3.0 ± 1.75
	H[a]	2.4 ± 1.33	0.4 ± 0.25	1.4 ± 1.43	1.4 ± 1.43
A. arenaria (deg.)	A[a]	1.4 ± 1.43	0	8.6 ± 5.47	1.4 ± 1.43
	H[a]	8.6 ± 6.80	0	1.4 ± 1.43	1.4 ± 1.43
C. arenaria	A[b]	40.0 ± 14.75	20.0 ± 11.07	5.7 ± 2.33	36.2 ± 17.34
	H[a]	6.1 ± 5.58	0	0	4.5 ± 1.49

Numbers ± SE of *Tylenchorhynchus ventralis* and *Tylenchorhynchus microphasmis*/*Tylenchorhynchus nanus* (N 100 g^{-1} dry soil) in the root zone of vigorous and degenerate *Ammophila arenaria* (in April vigorous *A. arenaria* $n = 8$, all other $n = 4$) and of *Carex arenaria* ($n = 4$). Samples were taken at two locations (Autostrand (A) and Haringvlietdam (H)) on four sampling dates. Different letters behind location abbreviations refer to significant differences in density of *T. microphasmis*/*T. nanus* among plants and locations, but not among sampling dates (Mann–Whitney U with Bonferroni correction $P < 0.05$).

density of *T. ventralis* was equally low on vigorous and degenerate *A. arenaria* and on *C. arenaria*, and did not significantly vary during the year (Table 1; $H_{1\,(\text{location})} = 0.98$, $H_{2\,(\text{plant})} = 2.30$, $H_{3\,(\text{time})} = 6.58$, all n.s.). The density of the combination of *T. microphasmis* and *T. nanus* (which we did not distinguish from each other in the field samples) on both plant species differed between the two locations (Table 1; $H_{2\,(\text{plant} \times \text{location})} = 10.25$, $P < 0.01$). At Haringvlietdam, the density was similar on both plant species. However, at Autostrand densities were higher on *C. arenaria* than on *A. arenaria*. At both locations, densities did not differ between vigorous and degenerate *A. arenaria*. Densities of *T. microphasmis* and *T. nanus* varied significantly during the year (Table 1; $H_3 = 9.13$, $P < 0.05$), but Mann–Whitney U test with Bonferroni correction did not reveal significant differences between sampling dates.

3.2. Competition experiment

The multiplication of *T. ventralis* on both plant species decreased with increasing inoculum densities (Fig. 1; Wald 21.4, $P < 0.001$), pointing at density-dependent intraspecific competition. The response to intraspecific competition was curvilinear (Tv × Tv; Wald 10.1, $P < 0.01$). Addition of *T. microphasmis* significantly hampered the multiplication of *T. ventralis*, but only on *A. arenaria* (plant × Tm; Wald 36.3, $P < 0.001$). The multiplication of a monospecific population of *T. ventralis* was 20–40 times lower on *C. arenaria* than on *A. arenaria* (Fig. 1; Wald 277.3, $P < 0.001$). In the case of *C. arenaria*, addition of *T. microphasmis* had a positive effect on the multiplication of *T. ventralis* (Fig. 1; interaction plant × Tm; Wald 36.3, $P < 0.001$). Thus, the interaction with *T. microphasmis* turned from interspecific competition on the good host into facilitation towards *T. ventralis* on the poor host.

The multiplication of *T. microphasmis* did not significantly differ between the two plant species (Fig. 1). The multiplication of *T. microphasmis* on both plant species decreased with increasing inoculum densities (Fig. 1; Wald 15.0, $P < 0.001$), pointing at density-dependent intraspecific competition. The effect of intraspecific competition was curvilinear (Tm × Tm; Wald 6.1, $P < 0.05$). The effect of intraspecific competition was stronger on *A. arenaria* than on *C. arenaria* (Fig. 1; plant × Tm; Wald 21.3, $P < 0.001$). On both plant species, addition of *T. ventralis* did not significantly affect the multiplication of *T. microphasmis* (Fig. 1). Therefore, the effect of interspecific competition is asymmetric and the results depend on the nematode species, as well as the suitability of the host plant under consideration.

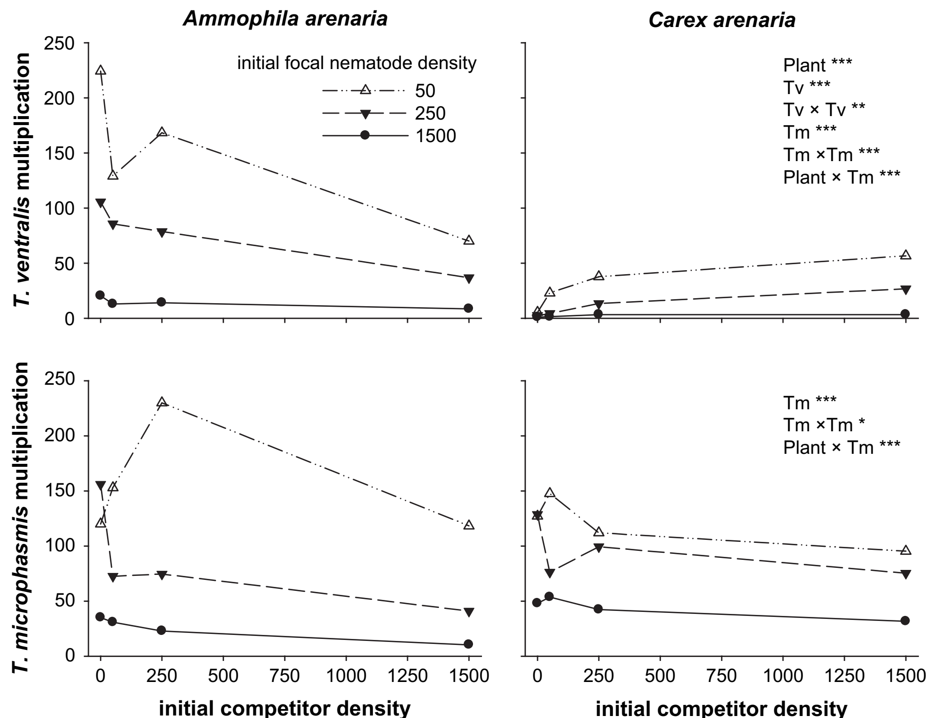

Fig. 1. Competition experiment. Multiplication (final nematode density divided by inoculum density) of *Tylenchorhynchus ventralis* (Tv; upper row) and *Tylenchorhynchus microphasmis* (Tm; lower row) 12 weeks after inoculation. Different densities (0, 50, 250 and 1500 per pot) of each nematode species were concomitantly added to *Ammophila arenaria* (left) and *Carex arenaria* (right) ($n = 6$). Statistics refer to significant results of a Generalized Linear Model testing effects of plant species and densities of the two nematode species on multiplication of each nematode species (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Addition of *T. ventralis* significantly decreased shoot biomass of both *A. arenaria* and *C. arenaria* (Table 2; Wald 96.8, $P<0.001$). However, shoot biomass of *A. arenaria* was reduced more than that of *C. arenaria* (Wald 34.9, $P<0.001$). Addition of *T. microphasmis* also significantly decreased shoot biomass of both plant species (Table 2; Wald 15.1, $P<0.001$). In contrast to *T. ventralis*, the effect of *T. microphasmis* on shoot biomass of *A. arenaria* was smaller than the effect on *C. arenaria* (Wald 9.4, $P<0.01$). The interaction between the two nematode species and the three-way interaction were not significant and were removed from the analysis (Crawley, 2005).

Only *C. arenaria* formed rhizomes, so that the analysis of rhizome biomass did not include plant species as a factor. Both *T. ventralis* (Wald 35.5, $P<0.001$) and *T. microphasmis* (Wald 27.2, $P<0.001$) had a negative effect on rhizome biomass of *C. arenaria* (Table 2). The interaction between the two nematode species was not significant and was removed from the analysis (Crawley, 2005).

Addition of *T. ventralis* (Wald 61.4, $P<0.001$) and of *T. microphasmis* (Wald 34.2, $P<0.001$) decreased root biomass of both plant species (Table 3). However, in line with the effect on shoot biomass, the effect of *T. ventralis* was greater on *A. arenaria* than on *C. arenaria* (Wald 31.4, $P<0.001$). The three-way interaction between plant species, *T. microphasmis* density and *T. ventralis* density was significant. On *A. arenaria*, adding *T. ventralis* further reduced root biomass of plants with high inoculation levels of *T. microphasmis*. In contrast, adding *T. microphasmis* did not further reduce root biomass of *A. arenaria* inoculated with 1500 *T. ventralis*.

3.3. Split-root experiment

When the roots of each plant were divided over two separate compartments, the presence or the identity of nematodes in one root compartment did not significantly influence the multiplication of *T. microphasmis* and of *T. ventralis* in the adjacent root compartment (Table 4; $F_{2,48}=0.152$, $P=0.86$). The interaction between nematode species and plant species was significant ($F_{1,48}=50.87$, $P<0.001$), so that final numbers of each nematode species differed between plant species. Six weeks after inoculation, numbers of *T. microphasmis* were significantly higher on *C. arenaria* than on *A. arenaria* (Table 4). In contrast, but similar to the competition experiment, numbers of *T. ventralis* were significantly lower on *C. arenaria* than on *A. arenaria* (Table 4). However, numbers of *T. ventralis* on *C. arenaria* tended to increase when *T. microphasmis* was added to the adjacent root compartment (Table 4; $F_{2,48}=2.44$, $P=0.098$), so that a systemic effect cannot completely be ruled out.

Table 2
Competition experiment

Nematode species	*N* per pot	Biomass (g dm per pot) ± SE		
		A. arenaria	*C. arenaria*	
		Shoot	Shoot	Rhizome
Tv	0	5.26 ± 0.13	3.35 ± 0.08	1.44 ± 0.06
	50	5.20 ± 0.11	3.42 ± 0.06	1.60 ± 0.07
	250	5.20 ± 0.11	3.41 ± 0.06	1.26 ± 0.05
	1500	4.00 ± 0.14	3.10 ± 0.09	1.11 ± 0.04
Tm	0	5.08 ± 0.16	3.40 ± 0.06	1.43 ± 0.05
	50	4.76 ± 0.17	3.41 ± 0.05	1.40 ± 0.07
	250	4.95 ± 0.15	3.56 ± 0.06	1.46 ± 0.07
	1500	4.87 ± 0.17	2.90 ± 0.08	1.11 ± 0.06

Influence of the added number per pot of *Tylenchorhynchus ventralis* (Tv) and *T. microphasmis* (Tm) on the dry biomass (g dm per pot) of shoot of *Ammophila arenaria* and shoot and rhizome of *Carex arenaria* ($n=24$).
Results of GLM: shoot: plant ***, Tv ***, Tm ***, plant × Tv ***, plant × Tm **; rhizome: Tv ***, Tm *** (** $P<0.01$, *** $P<0.001$).

Table 3
Competition experiment

Plant species	Tv	Root biomass (g dm per pot) ± SE			
		Tm (*N* per pot)			
		0	50	250	1500
A. arenaria	0	1.51 ± 0.04	1.02 ± 0.14	1.06 ± 0.14	0.92 ± 0.07
	50	1.18 ± 0.11	1.02 ± 0.07	0.93 ± 0.11	0.70 ± 0.06
	250	0.93 ± 0.06	0.81 ± 0.08	0.62 ± 0.04	0.53 ± 0.04
	1500	0.31 ± 0.03	0.35 ± 0.02	0.38 ± 0.03	0.29 ± 0.02
C. arenaria	0	1.86 ± 0.28	1.88 ± 0.16	1.66 ± 0.13	1.25 ± 0.09
	50	1.64 ± 0.09	1.83 ± 0.17	1.81 ± 0.24	1.79 ± 0.16
	250	1.67 ± 0.09	1.72 ± 0.08	1.74 ± 0.07	1.46 ± 0.14
	1500	1.76 ± 0.14	1.82 ± 0.13	1.48 ± 0.10	1.20 ± 0.14

Influence of the added number per pot of *Tylenchorhynchus ventralis* (Tv) and *T. microphasmis* (Tm) on the root dry biomass (g dm per pot) of *Ammophila arenaria* and *Carex arenaria* ($n=6$).
Results of GLM: plant ***, Tv ***, Tm ***, plant × Tv ***, plant × Tv × Tm * (* $P<0.05$, *** $P<0.001$).

Independent of the addition treatment, the dry biomass of the roots in the two compartments was not significantly different, so that total root biomass is presented. The nematode treatments did not result in significant differences in shoot and in total root dry biomass. The shoot and root dry biomass ± SE of *A. arenaria* ($n=24$) was 0.70 g ± 0.046 and 0.28 g ± 0.022, respectively, and the shoot and (root + belowground shoot) biomass ± SE of *C. arenaria* ($n=36$) was 0.61 g ± 0.016 and 0.86 g ± 0.033, respectively.

3.4. Attraction experiment

The fraction of *T. microphasmis* and *T. ventralis* that chose either of two quadrants with or without *A. arenaria* or *C. arenaria* seedlings significantly increased until 16 and 19 h after inoculation to 0.17 and 0.24, respectively (effect of time $F_{4,\ 208}=68.42$ and $F_{3,\ 186}=20.8$, respectively, $P<0.001$; data not shown). The presence of plants or the plant combination did not have a significant effect on the fraction of the added nematodes that made a choice for either of the two quadrants. For both nematode species, however, the presence of a plant and the plant species did influence the choice between the two quadrants of the Petri dish (Fig. 2). *T. ventralis* showed a preference for one of the two quadrants without a plant ($P<0.05$); however, this choice effect did not occur when both quadrants contained the same plant species ($P>0.05$). *T. ventralis* significantly preferred *A. arenaria* when the alternative choice involved no plant, or *C. arenaria* (Fig. 2, $P<0.001$). However, *T. ventralis* did not prefer *C. arenaria* over a quadrant without

Table 4
Split-root experiment

Nematode species	Addition	*A. arenaria*	*C. arenaria*
		N pot $^{-1}$	*N* pot $^{-1}$
T. ventralis	No	2003 (681–5893)	173 (79–377)
	Same	1623 (268–9840)	303 (212–432)
	Other	974 (92–10271)	415 (182–951)
T. microphasmis	No	1321 (760–2294)	2791 (2298–3389)
	Same	1008 (509–1993)	2184 (1484–3215)
	Other	828 (285–2404)	2380 (1909–2968)

The influence of nematode addition (no, same or the other species) to the adjacent root compartment on final numbers of *Tylenchorhynchus ventralis* and *T. microphasmis* 6 weeks after inoculation to *Ammophila arenaria* ($n=4$) and to *Carex arenaria* ($n=6$). The numbers are backtransformed means of $\ln(x+1)$-transformed numbers and 95% confidence limits are given between brackets.
Interaction plant species × nematode species $F_{1,48}=50.9$, $P<0.001$; plant species × nematode species × addition $F_{2,48}=1.12$, $P=0.34$.

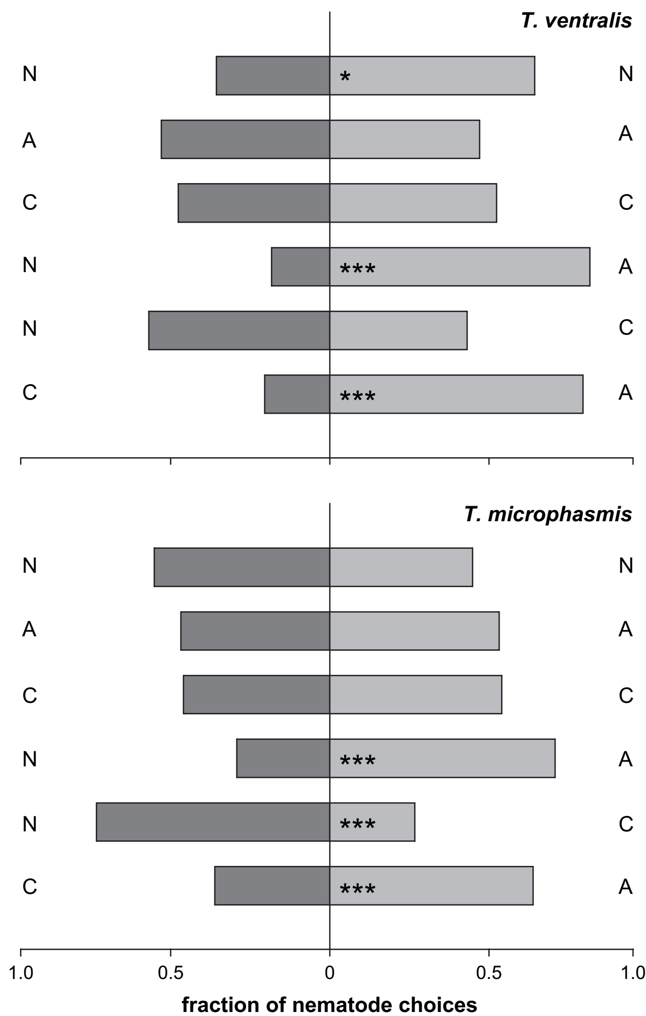

Fig. 2. Attraction experiment. The fraction of choices of *Tylenchorhynchus ventralis* and *Tylenchorhynchus microphasmis* between two quadrants of a Petri dish, after 32 and 34 h, respectively. Each of the two quadrants contained no plant (N), *Ammophila arenaria* (A) or *Carex arenaria* (C). Asterisks indicate whether the nematode distribution of each of the treatments differed from a random distribution ($n = 11, 12, 12, 12, 12$ and 10 and $n = 10, 10, 9, 10, 10$ and 9 for *T. ventralis* and *T. microphasmis*, respectively, ranging from the top to the bottom of the figure; $^{*}P < 0.05$, $^{***}P < 0.001$).

a plant. *T. microphasmis* did not show a preference when offered a choice between quadrants without plants, or when offered a choice between compartments that contained the same plant species (Fig. 2). *T. microphasmis* showed a significant preference for *A. arenaria* when the alternative choice was no plant, or *C. arenaria* ($P < 0.001$). Unexpectedly, *T. microphasmis* avoided *C. arenaria* when the alternative choice was no plant (Fig. 2, $P < 0.001$), although this preference was not as clear at the other time points (data not shown).

4. Discussion

4.1. Nematode interactions

We showed that interactions between root-feeding herbivorous nematodes with comparable feeding strategies depended on the nematode species and on the plant species that were used as a host. For both nematode species, the effect of intraspecific interactions on nematode multiplication was stronger than the effect of interspecific interactions. Therefore, feeding niches on each plant species overlapped, but were not exactly the same. This would leave open the possibility for coexistence on the same plant species (Hutchinson, 1957).

When added alone to the dune grass *A. arenaria* and to the sedge *C. arenaria*, the reproduction of *T. microphasmis* was equally high. In contrast, when added alone to both plant species, the reproduction of *T. ventralis* was high on *A. arenaria*, but low on *C. arenaria*. When added together, the multiplication of *T. microphasmis* was not significantly affected, irrespective of the plant species that was used as a host. On the contrary, the effect of adding the other nematode species to *T. ventralis* depended on the plant species. When added together to *A. arenaria*, the good host, the multiplication of *T. ventralis* decreased. When added together to *C. arenaria*, the poor host, the multiplication of *T. ventralis* did not decrease, but enhanced. Thus, on *A. arenaria*, the presence of the two nematode species resulted in asymmetric competition, *T. microphasmis* suppressing *T. ventralis*. On *C. arenaria*, *T. microphasmis* did not suppress, but facilitated multiplication of *T. ventralis*. The facilitation may be a result of a mechanical or physiological change of the root system caused by nematode feeding (Eisenback, 1993), which we attempted to unravel in subsequent experiments.

4.2. Mechanisms of nematode interactions

In the split-root experiment, addition of nematodes to one half of the root system did not lead to a significant change in nematode multiplication in the other root half. However, a slight systemic change in the roots of either *A. arenaria* or *C. arenaria* influencing the interaction between *T. microphasmis* and *T. ventralis* cannot be ruled out completely. On *C. arenaria*, numbers of *T. ventralis* tended to increase when *T. microphasmis* was present in the other half of the root system ($P = 0.098$). In the competition experiment, this increase was significant and the effects in both experiments pointed into the same direction of facilitation. As the duration of the split-root experiment was shorter than of the competition experiment and time is known to influence the results of interaction experiments (Eisenback, 1993), systemic effects might show up when time proceeds.

In a two-choice test with only one plant species present as well as with both plant species, both *T. microphasmis* and *T. ventralis* were attracted to *A. arenaria* but not to *C. arenaria*. As *T. ventralis* reproduced poorly on *C. arenaria*, unattractiveness of this plant species was to be expected. However, it was unexpected that *T. microphasmis* was attracted to *A. arenaria* and repelled by *C. arenaria*, while both plant species were equally suitable as a host. Therefore, there is not a clear link between preference for and performance on a host plant. Only 19–24% of the added nematodes made a choice for either of the two quadrants, irrespective of the presence of plants. This migration fraction was similar to other choice experiments with root-feeding nematodes (Lavallee and Rohde, 1962), indicating that either only a fraction of the added nematodes was active or that the attraction or repellence was not pronounced. Root-feeding nematodes are sensitive to small concentration differences (Prot, 1980; Perry, 2005), enabling them to orient their movement to the source of the attractant. The distance between the inoculation point and the plants was small enough to allow diffusion of short-distance attractants (Lavallee and Rohde, 1962). However, as root attractiveness depends on the growth rate of the plant (Prot, 1980), the presence of the plants in a nutrient-poor environment like water agar may have limited plant growth and consequently attractiveness. In soil, root exudates travel slower and, depending on the solute, over smaller distances than in agar, so that the results may not be simply extrapolated to field conditions (Watt et al., 2006). Nematode attraction to plant roots might change through

feeding by other root-feeding nematode species, which has not been examined in the present study.

4.3. Effects on plant biomass

Addition of *T. ventralis* reduced the shoot biomass of *A. arenaria* more than did *T. microphasmis*, whereas for *C. arenaria* the effect was the reverse. On *C. arenaria*, the small growth reducing effect of *T. ventralis* may be due to low host suitability, so that nematode numbers, as well as their effect size remained low. On *C. arenaria*, the addition effects of *T. microphasmis* and *T. ventralis* on plant biomass did not depend on each other. In contrast, the addition effects on root biomass of *A. arenaria* were interactive. Adding *T. ventralis* further reduced root biomass of plants that were exposed to high inoculation levels of *T. microphasmis*. However, addition of *T. microphasmis* to plants with high inoculation levels of *T. ventralis* did not further reduce root biomass.

In the split-root experiment, the plants were larger at the time of inoculation than the plants in the competition experiment. Moreover, the split-root experiment lasted shorter than the competition experiment. Both different conditions may explain why addition of nematodes did not significantly affect plant biomass within the time span of the split-root experiment. In the field, densities of both *Tylenchorhynchus* spp. are much lower than the densities used in these experiments under controlled conditions. Therefore, in the field direct growth inhibition of *A. arenaria* and *C. arenaria* by the two nematode species will be insignificant.

4.4. Implications of interactions in the field

Host suitability does not explain why in the field on both plant species the density of both nematodes, especially of *T. ventralis*, is so low. On the contrary, specificity experiments in controlled conditions suggest high densities on *A. arenaria* (De Rooij-van der Goes, 1995; Brinkman et al., 2004) and low densities or absence on *C. arenaria* (this study). In controlled conditions, competition with other root-feeding nematodes like *T. microphasmis* (this study) and endoparasites (Brinkman et al., 2004) limited the multiplication of *T. ventralis* on *A. arenaria*. However, competition did not decrease the density of *T. ventralis* to levels that are normally observed in the field (De Rooij-van der Goes et al., 1995; Van der Stoel et al., 2002). Recently, top-down control by microorganisms has been shown to limit the abundance of *T. ventralis* on *A. arenaria* (Piśkiewicz et al., 2007). On *C. arenaria*, however, in the absence of *T. microphasmis*, *T. ventralis* is barely able to sustain a population. Therefore, our results suggest that facilitation by *T. microphasmis* contributes to persistence, if only in low densities, of *T. ventralis* on *C. arenaria*. Processes like competition and facilitation, as demonstrated in our study, may contribute to the spatially aggregated distribution patterns that nematodes typically show (Ettema and Wardle, 2002).

In our relatively simple study system, focusing on two ectoparasitic root-feeding nematode species and two host plant species, nematode interactions resulted in two types of effects: asymmetric competition and facilitation. As most natural or agricultural ecosystems consist of more than two plant and/or nematode species, interactions will be much more complex, including bottom-up, horizontal and top-down effects. As a result, the population dynamics of root-feeding nematodes will be influenced not only by the suitability of the host plant, but also by the presence and identity of surrounding plant species (De Deyn et al., 2004; Schroeder et al., 2005), other root-feeding nematode species and natural enemies. Enhancing our knowledge on nematode interactions in natural ecosystems may help to further understand and manage the abundance of root-feeding nematodes in agricultural or horticultural ecosystems (Van der Putten et al., 2006).

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