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Coexistence of entomopathogenic nematode species (Steinernematidae and Heterorhabditidae) with different foraging behavior

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Summary – We studied the interactions between two entomopathogenic nematode species each with a different foraging behavior in the presence of two hosts at different soil depths. One week after nematode inoculation, hosts were added to the soil and additional hosts added at 30-day intervals. The densities of infective juveniles of the two nematode species were monitored over 90 to 180-day periods in soil samples or directly in the experimental containers using wax moth larvae as baits. Host placement in the soil strongly influenced the outcome of the competition between the sit-and-wait forager (ambusher) *Steinernema carpocapsae* and active forager (cruiser) *S. glaseri*. When hosts were introduced at 2 and 10 cm depths, few *S. carpocapsae* were recovered at 60 days and onwards after the first hosts had been introduced. *S. glaseri*, on the other hand, was not adversely affected by the presence of *S. carpocapsae*. When hosts were introduced at 0 and 10 cm depths, *S. carpocapsae* persisted in the presence of *S. glaseri* at significantly lower numbers than when it was alone, whereas *S. glaseri* was not significantly affected by the presence of *S. carpocapsae*. Numbers of recovered *S. glaseri;* however, tended to decrease after 30 – 60 days, even in the absence of its competitor. We postulate that this decrease was because of its high susceptibility to intraspecific competition. In the combination of *S. carpocapsae* and the active forager *Heterorhabditis bacteriophora, S. carpocapsae* dominated the system during the first 90 days, and *H. bacteriophora* dominated during the last 60 days. In the laboratory, an ambusher and a cruiser entomopathogenic nematode species can coexist. In the field, many other factors will influence the population dynamics, and the higher diversity should promote the coexistence of entomopathogenic nematode species with different foraging behavior.

Résumé – ***Coexistence des espèces de nématode entomopathogènes (Steinernematidae et Heterorhabditidae) ayant différents comportements de recherche de l'hôte.*** Nous avons étudié les interactions entre deux espèces de nématodes entomopathogènes ayant différents comportements de recherche de l'hôte (actif *vs* passif), en présence de deux hôtes placés à différentes profondeurs dans le sol. Des hôtes étaient ajoutés au sol à intervalle de 30 jours. Les densités des larves infestantes des deux espèces ont été suivies sur des périodes de 90 à 180 jours dans des échantillons de sol ou directement dans les récipients expérimentaux avec des larves de *Galleria*. La position des hôtes s'est avérée prépondérante en ce qui concerne la compétition entre le comportement passif de *Steinernema carpocapsae* et le comportement actif de *S. glaseri*. Avec les hôtes placés à 2 et 10 cm de profondeur, *S. glaseri* dominait nettement. Avec les hôtes placés à 0 et 10 cm de profondeur, *S. carpocapsae* a persisté en présence de *S. glaseri* mais le nombre des larves infestantes a été significativement réduit. Par contre, *S. glaseri* n'a pas été influencé significativement par la présence de *S. carpocapsae*. Le nombre des *S. glaseri* avait tendance à diminuer après 30-60 jours, même en l'absence de compétiteur. Nous supposons que cette diminution est liée à sa sensibilité à la compétition intraspécifique. Pour la combinaison de *S. carpocapsae* et *Heterorhabditis bacteriophora, S. carpocapsae* dominait le système pendant les premiers 90 jours alors que *H. bacteriophora* dominait le système pendant les derniers 60 jours. Au laboratoire, deux espèces de nématodes entomopathogènes, l'une à comportement passif et l'autre à comportement actif, peuvent coexister. En champ, de nombreux facteurs influencent la dynamique des populations et favorisent la coexistence d'espèces de nématodes entomopathogènes à comportements de recherche de l'hôte différents.

Key-words : *Steinernema, Heterorhabditis,* soil ecology, intraspecific competition, interspecific competition.

The significance of interspecific competition has been a controversial subject for decades (Schoener, 1982), but experimental field studies (Schoener, 1983; Connel, 1983) have confirmed that competition is a common phenomenon in nature. Competing species may coexist in an ecological community if mechanisms exist that slow down the rate of competitive exclusion or buffer interspecific competition. Examples for these mechanisms are *i*)seasonal variation in environmental conditions, resource abundance and activity patterns of competitors (Chesson & Huntley, 1989; Carton *et al.*, 1991); *ii)* differential use of a common resource (Schlyter & Anderbrandt, 1993); *iii)* higher susceptibility of an intrinsically superior competitor to predator attack (Norrdahl & Korpimäki, 1993); *iv)* different foraging strategies bringing about temporal partitioning (Kotler *et al.*, 1993), different intensities in resource exploitation (Schröpfer & Klenner-Frings, 1991; Perfecto, 1994) or different breadth of trophic niches (Bergallo & Rocha, 1994).

Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* (Rhabditida : Steinernematidae and Heterorhabditidae) may compete for the same host in nature. They are widely distributed in soils throughout the world (Kaya, 1990). On many occasions more than one species have been isolated from the same sampling site (Beavers *et al.,* 1983; Akhurst *et al.,* 1992; Stuart & Gaugler, 1994; Campbell *et al.,* 1995). Although the sampling methods usually were not designed to determine whether they actually occured in the same spot, these findings suggest that two entomopathogenic nematode species may coexist but little is known about their interactions.

Coexistence of entomopathogenic nematode species may, in part, be a consequence of their foraging behavior. The foraging behaviors of the infective third-stage juvenile (IJ) of these nematodes range from one extreme of a sit-and-wait strategist (ambusher) to the other extreme of an active foraging strategist (cruiser) (Campbell & Gaugler, 1993). *Steinernema carpocapsae* is an ambusher that nictates (Kondo & Ishibashi, 1986), tends to stay near the soil surface and does not disperse far (Moyle & Kaya, 1981), is unresponsive to host cues (Lewis *et al.,* 1992), and is adapted to infect mobile hosts on the soil surface (Campbell & Gaugler, 1993). *S. glaseri* and *Heterorhabditis bacteriophora,* on the other hand, are cruisers that disperse actively and are distributed more uniformly in the soil (Georgis & Poinar, 1983; Schroeder & Beavers, 1987), respond strongly to host cues (Lewis *et al.,* 1992), and are adapted to infect sedentary hosts (Campbell & Gaugler, 1993).

The IJs of these nematodes carry cells of a species specific bacterial symbiont in the genus *Xenorhabdus* for *Steinernema* and *Photorhabdus* for *Heterorhabditis* in their intestine (Boemare *et al.,* 1993*a*). When IJs find a suitable insect host, they enter it through natural openings and penetrate into the host's hemocoel. The bacteria are released, propagate in the hemocoel, and cause a lethal septicemia within 2 days. The infection by the nematode/bacterium complex is associated with a typical change in color of the host cadaver. In the greater wax moth, *Galleria mellonella,* a creamy color is typical for the *S. carpocapsae*/*X. nematophilus* complex, a dark rusty color for the *S. glaseri*/*X. poinarii* complex, and an orange to red color for the *H. bacteriophora*/*P. luminescens* complex (Woodring & Kaya, 1988). The nematodes feed on the bacteria and the host tissue, go through two or three generations, and finally emerge from the depleted host cadaver as IJs (Poinar, 1990). If steinernemetids and heterorhabditid coinfect a host, only that species whose symbiotic bacterium successfully colonizes the host will reproduce (Akhurst, 1983; Alatorre-Rosas & Kaya, 1990, 1991). Within a genus, nematode species can feed on the bacteria of other species (Dunphy *et al.,* 1985) and two steinernematid species can reproduce within one host cadaver (Kondo, 1989; Koppenhöfer *et al.,* 1995).

Our objective was to study interactions between two entomopathogenic nematode species each with a different foraging behavior under controlled conditions. The occurence of two hosts at different soil depths should allow the nematodes to coexist if each of them would predominantely infect hosts at different soil depths according to their typical foraging behavior. In a small arena with two hosts present at different soil depths, we studied the effect of host depths on the interactions between an ambusher and a cruiser nematode species (experiment 1 and 2) during 4 months. A larger arena and the presence of multiple hosts/soil depth was expected to stabilize the system when we repeated the study during a prolonged observation period (experiment 3).

Material and methods

All experiments were conducted at 20 ± 1 °C, using a sandy loam soil (75 % sand, 18 % silt, 7 % clay; 0.3 % organic matter; pH 6.9) that had been autoclaved at least 2 weeks before use. The final soil moisture after inoculation with nematode suspension was 13 % (w/w) moisture (– 6 kPa water potential). In all experiments, the containers were covered with plastic bags to reduce moisture loss, and the soil moisture was monitored using the filter-paper method for the determination of soil matric potential (Hamblin, 1981). *S. carpocapsae* All strain, *S. glaseri* NC strain, and *H. bacteriophora* NC1 strain were cultured in last instar larvae of the greater wax moth, *Galleria mellonella* (Northern Bait, Chetek, WI), and the IJs harvested from White traps and stored in sterilized distilled water at 10 °C (Woodring & Kaya, 1988) for 5-21 days before use.

To monitor trends in IJ populations in the soil, we used live insects as baits (Bedding & Akhurst, 1975). Because the proportion of IJs within a nematode population able to infect a host changes over time (Fan & Hominick, 1991), the number of nematodes recovered with bait insects does not give an exact picture of the actual number present in the soil. The changes in proportion of infectives, however, are not very large (Bohan & Hominick, 1994) and in the present study we were interested in population trends rather than absolute numbers.

Experiment 1

The general experimental plan was to place 800 ml of moist soil into 1-dm^3 styrofoam cups (13 cm soil height) followed by the addition of 10 ml of sterilized distilled water to the soil surface containing no IJs, 4750 IJs of *S. carpocapsae* or *S. glaseri,* or 2375 IJs of each nematode species. After 1 week (sampling day 0), the first set of cups was destructively sampled by cutting them horizontally at 5 cm soil depth. From both the 0-5 cm and the 5-13 cm sections, two 100 cm^3 soil samples were taken. Each soil sample was evaluated by baiting with five wax moth larvae. The larvae were recovered 4 days

later, rinsed in tap water, dissected in a 0.5 % pepsin solution and incubated for 2 h at 37 °C to digest the insect's tissues (Mauleon *et al.,* 1993) before counting the number of nematodes that penetrated into the insects. Meanwhile, the samples were baited for an additional 4 days and processed as above.

After the first set of cups was sampled, the remaining cups were treated as indicated in Table 1. Thus, one set of cups that had been inoculated with one nematode species only received no wax moth larvae, whereas all remaining cups that had been inoculated with no nematodes or one or two nematode species received two wax moth larvae as nematode hosts which were added at 0 and 10 cm or 2 and 10 cm depth. At 0 cm depth, the larva was placed directly on the soil surface; at 2 cm depth, the larva was placed in a hole (1.5 cm diam) bored to depth with a cork borer and the soil placed back on top of it; and at 10 cm depth, the larva was introduced from the side of the cup through a hole bored with a cork borer at that depth and the soil replaced. Each time the borer was used, it was decontaminated with hot water within a treatment and a different borer was used between treatments. Further destructive sampling was done 30, 60, 90, and 120 days after the first hosts were introduced. After each sampling time except 120 days, two new insects were added to each remaining cup as described above.

Table 1. *Summary of experimental design for coexistence of two entomopathogenic nematode species in soil.*

Nematode species	Depth of hosts in soil (cm)	Treatment code	Nematode inoculum	No. of replicates
EXPERIMENT 1				
–(1)	0 and 10	–	–	3
–(1)	2 and 10	–	–	3
Sc alone	no hosts	C(-)	4,750	3
Sc alone	0 and 10	C(0 + 10)	4,750	5
Sc alone	2 and 10	C(2 + 10)	4,750	5
Sg alone	no hosts	G(-)	4,750	3
Sg alone	0 and 10	G(0 + 10)	4,750	5
Sg alone	2 and 10	G(2 + 10)	4,750	5
Sc and *Sg*	0 and 10	CG(0 + 10)	2,375/species	5
Sc and *Sg*	2 and 10	CG(2 + 10)	2,375/species	5
EXPERIMENT 2				
Sc alone	0 and 10	C(0 + 10)	4,750	10
Sg alone	0 and 10	G(0 + 10)	4,750	10
Sc and *Sg*	0 and 10	CG(0 + 10)	2,375/species	10
EXPERIMENT 3				
–(1)	0 and 10	–	–	2
Sc alone	no hosts	C(-)	53,400	3
Sc alone	0 and 10	C(0 + 10)	53,400	5
Sg alone	no hosts	G(-)	53,400	3
Sg alone	0 and 10	G(0 + 10)	53,400	5
Hb alone	no hosts	H(-)	53,400	3
Hb alone	0 + 10	H(0 + 10)	53,400	5
Sc and *Sg*	0 and 10	CG(0 + 10)	26,700/species	5
Sc and *Sg*	0 and 10	CH(0 + 10)	26,700/species	5

(1) Treatment received no nematodes, but soil was checked for presence of nematodes. Because no nematodes were recovered, treatment was excluded from analysis.

EXPERIMENT 2

Our observations in experiment 1 made it necessary to investigate the number of IJs of each species that penetrated into each insect at 0 and 10 cm depth. Therefore, 1-dm^3 styrofoam cups were prepared, inoculated, and handled as in experiment 1. But hosts were placed only at 0 and 10 cm depths (Table 1). Insects were added as in the respective treatments in experiment 1, on days 0, 30, 60, and 90. Rather than taking subsamples of soil from each cup, the entire cup was used as a sampling unit. Thus, 4 days after adding insects on each sampling day, the introduced insects were recovered from the to be sampled cups, rinsed in tap water, dissected, digested, and the number of penetrated nematodes counted. Once sampled, the cups were discarded.

EXPERIMENT 3

Twenty-two-liter fiberglass containers (60 × 21 × 17.5 cm) were filled to a height of 15 cm with 15 dm^3 of soil. Then 50 ml of a nematode suspension in sterilized distilled water was applied to the soil surface. Treatments were as follows : no nematodes with hosts at 0 and 10 cm depths, each of the three species, *S. carpocapsae, S. glaseri,* or *H. bacteriophora,* with or without hosts added. In addition, *S. carpocapsae* and *S. glaseri* or *S. carpocapsae* and *H. bacteriophora* were combined in the presence of hosts (Table 1).

After 1 week (sampling day 0), 12 soil cores were sampled from each container with cork borers (2 cm diam). Decontamination of the cork borers between cups was conducted as in experiment 1. The upper and the lower 5 cm of the cores were separately pooled to give two 100 cm^3 samples per soil section while the sampling holes were refilled with sterilized moist soil. The soil samples were baited with five wax moth larvae and the larvae processed as in experiment 1 except that host cadavers with the typical color of *Heterorhabditis* infections were incubated at room temperature (23 ± 2 °C) for 2 more days to facilitate counting of this slower developing species.

After taking the first soil samples, ten wax moth larvae were set directly onto the soil surface of each container, and ten larvae were placed individually in 10 cm deep holes bored with a cork borer and then covered with the soil. Additional insects were added on days 30, 60, 90, 120, and 150, and soil were samples taken on days 30, 60, 90, 120, and 180.

Data analysis

The number of nematodes recovered/100 cm^3 soil in experiments 1 and 3 were analyzed in two ways : *(i)* we averaged the samples/soil section and the data were analyzed by depth and species, and *(ii)* we averaged all four soil samples/replicate and the data were analyzed by species. Before analyses, the data were $log_{10}(x + 1)$ transformed. The data from the first sampling day were analyzed separately with analysis of variance (general linear model), whereas the data from the remaining sampling days were analyzed with repeated measures analysis of variance (general linear model). Means were separated with Tukey's Studentized range test (Anon., 1988). In experiment 2, the number of nematodes established in the hosts at the two soil depths were analyzed *(i)* by day and species and *(ii)* by day, species, and depth using the t test (Anon., 1988). The time and treatment interactions for sampling days 30-90 were tested with a G test (Sokal & Rohlf, 1981). Untransformed means ± standard errors are presented. If differences among means were significant or not significant on several consecutive sampling days, we present the lowest or highest F and P values, respectively.

Results

In all experiments, there was no significant change in soil moisture and no nematodes were recovered from the non inoculated controls. Therefore, these controls were not included in the analysis. Wax moth larvae added to the soil surface moved around and some were observed partly in the soil. Insects introduced below the soil surface were never found on the surface, although some of the insects introduced at 2 cm depth had moved towards the surface as was observed in experiment 2.

S. glaseri in experiment 1 (Fig. 1), and *H. bacteriophora* and *S. glaseri* in experiments 1 and 3 (Figs 1,3) were uniformly distributed in the soil. *S. carpocapsae,* however, was recovered in higher numbers from the upper soil sections in both experiments but populations followed the same trends in both sections. We observed the same trends and differences between treatments, whether we analyzed the data by depth and species or by species only. We therefore present only the combined data, except for the graphical presentation of experiment 2.

Experiment 1

On the first sampling day (day 0), significantly less nematodes were recovered from the mixed treatments,

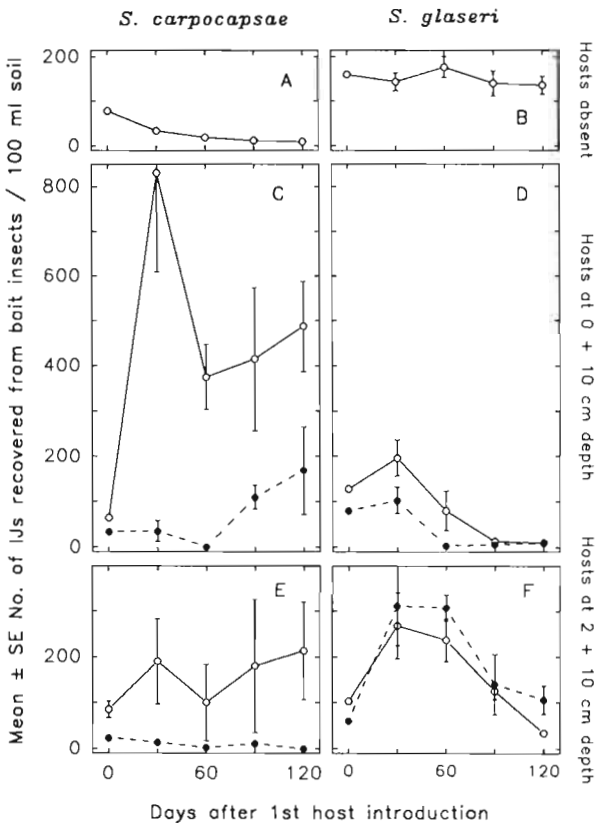

Fig. 1. *Nematodes recovered from cups with 800 ml of sterilized soil. The cups had been inoculated with 4750 infective juveniles of* Steinernema carpocapsae *or* S. glaseri *alone, or 2375 of each of these. After 0, 30, 60 and 90 days, two wax moth larvae were added as hosts, at 0 + 10 cm depth, or at 2 + 10 cm depth. A, C, E :* S. carpocapsae, *B, D, F :* S. glaseri; *A, B : in the absence of hosts; C, D : hosts at 0 + 10 cm; E, F : hosts at 2 + 10 cm;* (○) *in absence of competitor;* (●) *in presence of competitor (n = 5;20 °C).*

CG (0 + 10) and CG (2 +10) (see Table 1 for abbreviations), than from the other treatments for both *S. carpocapsae* ($F = 8.6$; df = 4,20; $P \leq 0.001$) and *S. glaseri* ($F = 35.0$; df = 4,20; $P \leq 0.001$). These differences were expected because the mixed treatments were inoculated with only half as many nematodes/species. Thereafter, in the absence of hosts the number of recovered *S. carpocapsae* IJs gradually declined [C(–)] (Fig. 1 A), whereas the number of recovered *S. glaseri* [G(–)] was stable throughout the experiment. In C(0 + 10) very high numbers of *S. carpocapsae* were recorded throughout the experiment (Fig. 1 C), whereas in G(0 + 10), *S. glaseri* was recovered at high numbers after 30 days and then declined (Fig. 1 D). In CG (0 + 10), the numbers of recovered IJs of *S. carpocapsae* and *S. glaseri* declined during the first 60 days. Thereafter, high numbers *S. carpocapsae* were recovered (Fig. 1 C), whereas the number of recovered *S. glaseri*

IJs remained very low (Fig. 1 D). In C(2 + 10), high numbers of *S. carpocapsae* were recovered throughout the experiment (Fig. 1 E), whereas in G(2 + 10) the numbers of recovered *S. glaseri* were high during the first 60 days but declined thereafter (Fig. 1 F). In the presence of the competitor [CG(2 + 10)], the numbers of recovered *S. carpocapsae* declined during the experiment and no *S. carpocapsae* could be recovered in most cups after 120 days (Fig. 1 E), whereas *S. glaseri* followed a similar trend to G(2 + 10) (Fig. 1 F).

Significant differences in numbers of *S. carpocapsae* recovered were observed at 30, 60, 90, and 120 days ($F \geq 7.4$; df = 4,20; $P \leq 0.001$). After 30-90 days, significantly more *S. carpocapsae* were recovered from the treatments to which insects had been added also on the soil surface, C(0 + 10) and CG(0 + 10), than from all other treatments. After 120 days, *S. carpocapsae* numbers were significantly lower in C(–) and in CG(2 + 10) than in all other treatments ($P \leq 0.05$). The repeated measures analysis of variance showed a significant interaction between time (conducted for day 30 through day 120, only) and treatment ($F = 3.0$; df = 12,48; $P \leq 0.01$) and found a linear trend between time and number of nematodes recovered in the treatments ($F = 5.0$; df = 4,20; $P \leq 0.01$).

No significant differences in numbers of recovered *S. glaseri* were found at 30 days ($F = 7.4$; df = 4,20; $P = 0.16$); but significantly different numbers of *S. glaseri* were recovered among treatments at 60, 90, and 120 days ($F \geq 12.7$; df = 4,20; $P \leq 0.001$). On these sampling days, recovery was higher in G(2 + 10) and CG (2 + 10) than in G(0 + 10) and CG(0 + 10) ($P \leq 0.05$). The repeated measures analysis of variance showed a significant interaction between time and treatment ($F = 3.4$; df = 15,48; $P \leq 0.001$) and found linear trends between time and number of nematodes recovered in the treatments ($F = 5.3$; df = 4,20; $P \leq 0.001$).

Experiment 2

On the first sampling day (day 0), the number of nematodes establishing in the hosts during 4 days of exposure in the experimental cups was significantly lower in the mixed treatment, CG(0 + 10), than in C(0 + 10) and G(0 + 10), respectively, for both *S. carpocapsae* ($t = 2.6$; df = 18; $P \leq 0.02$) and *S. glaseri* ($t = 8.7$; df = 18; $P \leq 0.001$). Again this was expected because of the lower inoculum/species in CG(0 + 10).

In C(0 + 10), *S. carpocapsae* establishment was very high at 30 and 60 days but dropped to the initial level on day 90 (Fig. 2 A). In the presence of the competitor, CG(0 + 10), *S. carpocapsae* was stable at a low rate throughout the experiment (Fig. 2 C). *S. glaseri* establishment rates followed very similar trends in the absence, G(0 + 10) (Fig. 2 B), and in the presence of the competitor, CG(0 + 10) (Fig. 2 D). Establishment of *S. carpocapsae* was higher in C(0 + 10) than in CG (0 + 10) at 30, 60, and 90 days ($t \geq 3.4$; df = 18;

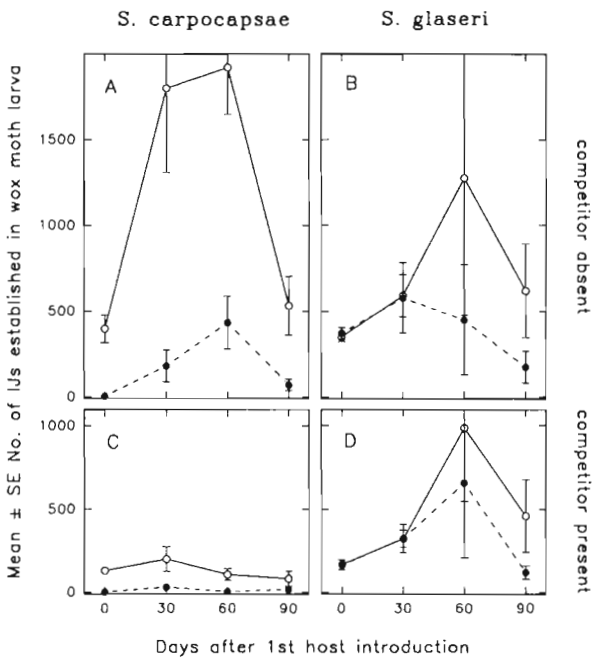

Fig. 2. *Nematodes recovered from two wax moth larvae placed for 96hr at 0 (○) and 10 cm depth (●) in cups with 800 ml of sterilized soil. The cups had been inoculated with 4750 infective juveniles of* Steinernema carpocapsae *or* S. glaseri *alone, or 2375 of each of these. After 0, 30, and 60 days, to each cup two wax moth larvae were added as hosts at 0 and 10 cm depth. A, C :* S. carpocapsae; *B, D :* S. glaseri; *C, D : in presence of competitor species (n = 10 cups; 20 °C).*

$P \leq 0.01$) whereas establishment of *S. glaseri* did not differ among treatments at 30, 60, and 90 days ($t \leq 1.1$; df = 180.0; $P > 0.3$). The G test showed a significant interaction between time and treatment in *S. carpocapsae* ($G_adj = 51.9$; df = 4; $P \leq 0.001$) and *S. glaseri* ($G_adj = 30.8$; df = 4; $P \leq 0.001$).

S. carpocapsae established in higher numbers in all insects at 0 cm [overall average : C(0 + 10) = 1163 ± 187; CG(0 + 10) = 131 ± 25] than at 10 cm depth [overal average : C(0 + 10) =208 ± 55; CG(0 + 10) = 17 ± 6] (n = 40). *S. glaseri* establishment, on the other side, was uniform at 0 cm [overall average : G(0 + 10) = 710 ± 223; CG(0 ± 10) = 485 ± 136] and 10 cm [G(0 + 10) = 436 ± 107; CG(0 + 10) = 347 ± 122] (n = 40). The highest number of nematodes recovered in one insect was 4.912 for *S. carpocapsae* in treatment C(0 + 10) on day 30, and 8.592 for *S. glaseri* in G(0 + 10), on day 60.

Experiment 3

On the first sampling day (day 0), significantly less nematodes were recovered from the mixed treatments than from the other treatments for *S. carpocapsae* ($F = 23.3$; df = 3,14; $P \leq 0.001$), *S. glaseri* ($F = 32.2$; df = 2,10; $P \leq 0.001$), and *H. bacteriophora* ($F = 35.9$;

df = 2,10; $P \leq 0.001$) as expected. Thereafter, the numbers of *S. carpocapsae* recovered decreased rapidly in C(–) and only few nematodes were recovered at 60 days and thereafter (Fig. 3 B). In C(0 + 10), very high numbers of *S. carpocapsae* were recovered at 30 days; thereafter, the numbers declined rapidly and stabilized at lower levels until the end of the experiment (Fig. 3 E). In CG(0 + 10), the number of recovered *S. carpocapsae* was similar on the first two sampling days but decreased during the next 60 days. Thereafter, it was stable at low numbers. At 180 days, this species was not recovered in three containers and in moderate numbers in two containers. *S. carpocapsae* followed a similar trend in CH(0 + 10) as in C(0 + 10) (Fig. 3 E).

Mean numbers of recovered *S. carpocapsae* were significantly different between treatments on days 30-180 ($F \geq 4.6$; df = 3.14; $P \leq 0.2$). On days 30-120, significantly more *S. carpocapsae* were recovered in C(0 + 10) and CH(0 + 10) than in CG(0 + 10) or C(–). On day 180, *S. carpocapsae* was recovered at significantly lower numbers in C(–) than in the other treatments ($P \leq 0.05$). The repeated measures analysis of variance showed no significant interaction between time and treatment (F = 1.4; df = 12,30; $P = 0.21$) but there was a quadratic decline in the number of nematodes recovered in the treatments (F = 4.7; df = 3,14; $P \leq 0.02$).

The number of recovered *S. glaseri* decreased gradually in G(–) but on day 180,10 % of the number of nematodes found on day 0 could be recovered (Fig. 3 A). In G(0 + 10), the number of recovered *S. glaseri* was similar on days 0 and 30, decreased gradually thereafter and only low numbers were recovered in all containers on day 180 (Fig. 3 D). In CG(0 + 10), *S. glaseri* followed a similar trend as in G(0 + 10) (Fig. 3 D). Differences among treatments in number of *S. glaseri* recovered were not significant on days 30-120 ($F \leq 4.0$; df = 2.10; $P > 0.08$), but were significant on day 180 (F = 8.2; df = 2.10; $P \leq 0.01$). At this time, the numbers recovered were higher in G(–) than in CG (0 + 10) ($P \leq 0.05$), whereas the numbers recovered in G(0 + 10) did not differ significantly from G(–) nor CG(0 + 10). The repeated measures analysis of

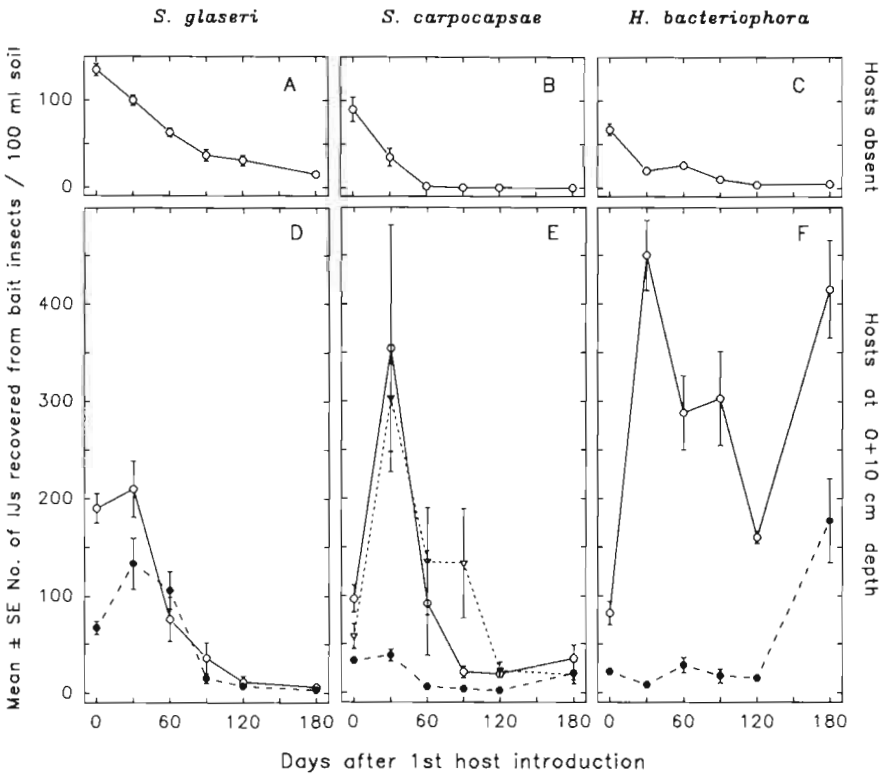

Fig. 3. *Nematodes recovered from containers with 15 dm³ of sterilized soil. The containers had been inoculated with 53 400 infective juveniles of* Steinernema carpocapsae, S. glaseri, *or* Heterorhabditis bacteriophora *alone, or 26 700 of* S. carpocapsae *and either of the two other species . At 30-day intervals, to each treatment container twenty wax moth larvae were set on the surface and placed 10 cm depth. A, D :* S. glaseri; *B, E* :* S. carpocapsae; *C, F :* H. bacteriophora; *A, B, C : in absence of hosts; D, E, F : in presence of hosts and absence* (○) *or presence* (●) *of competitor* (n = 5 for containers with insects, n = 3 for containers without insects; 20 °C).

(*● : in presence of *S. glaseri;* (▽) : in presence of *H. bacteriophora*.)

variance showed a significant interaction between time and treatment ($F = 3.8$; $df = 8,14$; $P \leq 0.02$) and a linear decline in the number of nematodes recovered in the treatments ($F = 8.1$; $df = 2,10$; $P \leq 0.01$).

In H(–), the numbers of *H. bacteriophora* recovered decreased rapidly during the first 30 days, but at 180 days the nematode were still recovered at 7 % of the initial number (Fig. 3 C). In H(0 + 10), the numbers recovered were very high throughout the experiment (Fig. 3 F). In CH(0 + 10), the numbers of *H. bacteriophora* recovered were stable at a low level during the first 120 days, but high at 180 days (Fig. 3 F). Significant differences in numbers of *H. bacteriophora* recovered among treatments were observed on days 30-180 ($F \geq 9.0$; $df = 2,10$; $P \leq 0.001$) and significantly more *H. bacteriophora* were recovered in H(0 + 10) than CH (0 + 10) or H(–). At 90-180 days, the number of recovered *H. bacteriophora* was also significantly higher in CH(0 + 10) than in H(–) ($P \leq 0.05$). The repeated measures analysis of variance showed a significant interaction between time and treatment ($F = 7.7$; $df = 8.14$; $P \leq 0.01$) and linear ($F = 46.0$; $df = 2,10$; $P \leq 0.001$) and quadratic ($F = 4.3$; $df = 2,10$; $P \leq 0.05$) trends in the number of nematodes recovered in the treatments.

Discussion

Dissection of the bait insects in experiment 2 showed that the densities of penetrating IJs in the hosts were often high enough to have a detrimental effect on progeny production. Each species showed a population reduction after a high density peak was recorded. *S. carpocapsae* had a greater population reduction and its numbers recovered more slowly than *H. bacteriophora*'s. *S. glaseri* was even more affected by intraspecific competition and tended to decrease during all experiments, even at relativity low densities, as shown by the repeated measures ANOVA. Our data can be explained by research conducted by Selvan *et al.* (1993) who showed that *H. bacteriophora* can tolerate higher densities of IJs penetrating in a host than *S. carpocapsae* before progeny production is adversely affected. *S. glaseri* seems to be even more susceptible to intraspecific competition than *S. carpocapsae* (Koppenhöfer & Kaya, 1995).

Although *S. glaseri* numbers initially increased more or less in the presence of hosts, at 90 days and onwards they were lower than in the no hosts treatments. Accordingly, where hosts were placed, many of them were infected by very high numbers of *S. glaseri* (experiment 2) and, instead of producing more progeny, these nematodes were actually depleting the number of IJs remaining in the soil. At this point, we do not have an explanation for *S. glaseri*'s failure to recover once it had reached low densities.

Our observations in experiment 1 show that the depth at which the hosts were placed was important for the outcome of the competition between *S. carpocapsae* and *S. glaseri*. If hosts were introduced at 2 and 10 cm depths, *S. glaseri* out competed *S. carpocapsae* to such an extent that the latter went extinct in some experimental cups (experiment 1). If hosts were introduced at 0 and 10 cm depth, *S. glaseri* was generally recovered in lower numbers and its numbers decreased faster in the presence of *S. carpocapsae* than when it was alone. The differences, however, were not significant and the numbers followed similar trends as in the absence of the competitor. *S. carpocapsae,* on the other hand, was significantly and adversely affected by the presence of the competitor but persisted at more or less reduced numbers compared with the *S. carpocapsae* alone treatments. Its populations were either stable at a low level (experiment 2) or showed an increasing trend after initially decreasing to very low densities (experiments 1 and 3).

The ambusher, *S. carpocapsae,* and the cruiser, *S. glaseri,* have the potential to coexist. *S. glaseri* is intrinsically superior to *S. carpocapsae* (Koppenhöfer *et al.,* 1995) due to its faster development and less specific association with its bacterial symbiont, enabling it to produce progeny in cadavers colonized by *S. carpocapsae*'s symbiont. In our system, however, *S. glaseri* populations were more or less limited which was, at least in part, because of its high susceptibility to intraspecific competition. Ongoing studies indicate that these two species may coexist better at lower IJ densities which enhance *S. carpocapsae*'s ability to out compete *S. glaseri* for hosts on the soil surface (Koppenhöfer, unpubl.). In the field, IJ densities would be limited by biotic and abiotic factors (Kaya, 1990).

The combination of *S. carpocapsae* and *H. bacteriophora* resulted in *S. carpocapsae* dominating numerically during the first 90 days. Because *H. bacteriophora* numbers did not decrease as in the treatment without hosts, we assume that it produced progeny to a limited extent. *S. carpocapsae* is intrinsically superior to *H. bacteriophora* probably because of the faster establishment of *S. carpocapsae*'s symbiotic bacterium (Alatorre-Rosas & Kaya, 1991; Boemare *et al.,* 1993 *b*). *H. bacteriophora,* on the other hand, is more motile which should enable it to out compete *S. carpocaspae* at the 10 cm soil depth. probably due to intraspecific competition, *S. carpocapsae* densities were low enough after 120 days to allow *H. bacteriophora* reproduction in a sufficient number of hosts to dominate the system for the last 60 days of the experiment as reflected by the significant quadratic trend in the repeated measures ANOVA. *S. carpocapsae* may have persisted in this system after the termination of the study due to its intrinsic superiority and better adaptation to infecting hosts on the soil surface. Thus, these two nematodes species can coexist in this system.

Our observations show that entomopathogenic nematode species with different foraging behavior may coexist in laboratory soil systems. In the field, however, population dynamics and competition will be influenced by many other environmental factors. One of the most im-

portant factors is probably the presence of different host species. These may not only differ in location, as simulated by different positions in our experiments, but also in their susceptibility to the different nematodes and suitability for their reproduction. The higher diversity of natural systems should be more likely to promote coexistence among entomopathogenic nematode species.

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