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Infection of the Entomopathogenic Nematode, *Steinernema carpocapsae,* as Affected by the Presence of *Steinernema glaseri*[1]

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Abstract: The infection behavior of *Steinernema carpocapsae* infective juveniles (IJ) was investigated in the presence and absence of *S. glaseri.* Mixed inoculation of *S. carpocapsae* with *S. glaseri* IJ significantly raised the nictation rates of *S. carpocapsae* IJ. Significantly more *S. carpocapsae* IJ migrated to the host insect in the mixed inoculation with *S. glaseri* IJ on agar plates. More *S. carpocapsae* IJ penetrated into the host insect placed 2 cm below the surface in the mixed inoculation with *S. glaseri* IJ. More *S. glaseri* than *S. carpocapsae* IJ penetrated into hosts placed 7 cm deep. Irrespective of host location, the male ratio of *S. carpocapsae* IJ established in the host body was always higher in the mixed inoculation with *S. glaseri* IJ.

Key words: behavior, competitor, entomopathogenic nematodes, infection behavior, mixed inoculation, nematode, nictation, sex ratio, *Steinernema carpocapsae, Steinernema glaseri.*

Steinernematid and heterorhabditid nematodes have become increasingly prominent biological control agents, especially against soil insect pests. Although they have a wide host range (Poinar, 1990), differences in their foraging strategies may limit their natural host range. *Steinernema carpocapsae,* which adopts an ''ambushing strategy,'' has been purported to be effective in parasitizing surface-dwelling insects, whereas *Steinernema glaseri,* which is called a ''cruiser,'' is generally more effective in killing subterranean sedentary insects such as *Popillia japonica* larvae (Kaya, 1990; Georgis and Gaugler, 1991; Lewis et al., 1992). Some steinernematid nematodes can feed on the bacteria of other species (Dunphy et al., 1985; Grewal et al., 1997), and two steinernematid species can reproduce within a single host cadaver (Kondo, 1989; Koppenhofer et al., 1995). The mixed application of entomopathogenic nematode species with different foraging behaviors can be used to control pests that differ in their location in soil and, as a result, in their availability to nematodes. Koppenhofer et al. (1996) studied the effect of host location on the interaction of *S. carpocapsae* and *S. glaseri,* and reported that infection of insects by these two species differed by soil depth. However, how infective behavior of these two species is affected when they are simutaneously applied in the same arena remains unclear. Our objective was to describe behavioral changes in *S. carpocapsae* infective juveniles (IJ) as affected by the presence of *S. glaseri* IJ.

Materials and Methods

Nematodes and hosts: Steinernema carpocapsae All strain was propagated at 25 °C on larvae of the greater wax moth, *Galleria mellonella,* according to Dutky et al. (1964) with a slight modification. The insect cadaver was placed on a piece of moist filter paper strip in a test tube. Dead or inactive IJ were removed by placement of the nematode suspension on a nylon sieve (30-μm aperture). Two hours later, active IJ that had passed through the sieve were collected and used for all experiments. The host insect, *G. mellonella,* was reared in beehives at 25 °C, and last-instar larvae weighing about 100 mg were used for experiments.

Nictation and infectivity: To avoid mixing of IJ that already had penetrated into the insect body with IJ penetrating later, early infection was established by the one-on-one method (Miller, 1989). A single IJ was put in each well of a 24-well tissue culture plate, then one insect was added to each well.

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Forty-eight hours later, insect cadavers that were killed by a single IJ were used as the infected host.

Nictation behavior was observed on sand (296–590-mm diam.) that had been autoclaved and dried before use. The final sand moisture after inoculation with 1,000 IJ was 13% (w/w) (Koppenhofer et al., 1996). The nictation rate was recorded in the following five treatments: (i) a single infected host placed at the bottom of the sand column in a styrofoam cup (4-cm height 3.25-cm diam.) filled with ca. 110 g moist sand, (ii) a single infected host placed on the surface of the sand column, (iii) a single uninfected host encased in a fine wire case (30 × 8 × 8 mm) at the bottom, or (iv) on the surface of the sand column, (v) control without a host insect in the sand column. After incubation at 25 °C for 30 min, 1,000 *S. carpocapsae* IJ in 1 ml of water were added to the top surface of the sand column, and a piece of nylon cloth sieve (125-µm aperture) was placed on the surface for the collection of nictating IJ (Ishibashi et al., 1994). Host insects for treatments ii and iv were placed on the nylon cloth. All cups were incubated in an insulated container to prevent moisture loss at 25 °C and were kept in the dark for 24 hours. Nictating IJ were collected by rinsing them from the cloth and were counted. The insect larva was taken from the sand column, rinsed with distilled water, and transferred to a petri dish. After incubation for an additional 48 hours, the insects were dissected to determine infection. This experiment was performed three times with five replicates for each treatment.

To determine the effect of the presence of *S. glaseri* on the nictation of *S. carpocapsae,* a mixture of 500 IJ each of *S. carpocapsae* and *S. glaseri* was added to the surface of sand columns with or without one *G. mellonella* larva. After 24 hours, the nictating IJ on the nylon cloth were collected and counted as described above. Inoculation with *S. carpocapsae* IJ alone without a host insect was used as a control. The experiment was performed three times with five replicates each time.

Interaction on an agar surface: A 2% agar solution was poured onto a transparent rectangular plastic plate (14 × 10 × 15 cm) and allowed to dry for 30 minutes. One *G. mellonella* larva was encased in a fine wire case (30 × 8 × 8 mm) at one side of the agar surface. Two hours later, a 10-µl mixed suspension of 50 *S. carpocapsae* and 50 *S. glaseri* IJ was sprayed onto the agar surface 5 cm from the host and incubated at 25 °C. Inoculations with 100 *S. carpocapsae* or 100 *S. glaseri* alone were conducted at the same time. After 5 hours, numbers of nematodes migrating toward the host were recorded. These numbers included nematodes, at least 4 cm away from the inoculation zone, including those that were on the host cuticle and had penetrated into the host. The experiment was performed three times with five replicates each time.

Interaction in a sand column: The interaction between the two nematode species was also observed in a sand column with 13% moisture (w/w). A 100-ml glass beaker (5.5-cm diam. 7.0-cm height) was used as a container. One *G. mellonella* larva was placed either at the bottom of the column (7 cm deep) or 2 cm below the surface. One milliliter of sterilized distilled water containing 1,000 IJ of *S. carpocapsae* or *S. glaseri,* or 1 ml with 500 IJ each of the two species, was distributed on the surface of the sand column. The containers were held in an insulated box to reduce moisture loss. After 24 hours of incubation at 25 °C, the insect was removed from the sand column and placed in a petri dish. The insects were dissected after an additional incubation of 48 hours, and the penetration of nematodes and their sex ratios were investigated. This experiment was performed three times with five replicates for each treatment.

Data analysis: The data were subjected to analysis of variance (ANOVA). If significant differences were found among the treatments, they were separated with Tukey's multiple range test at $P = 0.05$. Pairwise comparisons were made with a *t*-test at $P = 0.05$. The data are shown in means ± standard error of the mean.

Results

Nictation and infectivity: Nictation behavior of *S. carpocapsae* was influenced by the pres-

ence or absence of hosts, location of hosts, and infection status of hosts (infected or uninfected) (Fig. 1). Nictation rates of *S. carpocapsae* were significantly ($P < 0.05$) lower in all treatments containing hosts than in the no-host controls. Nictation rates were significantly higher ($P < 0.05$) in the presence of infected host than for uninfected hosts. Nictation rates were also significantly higher ($P < 0.05$) when the hosts were placed at the bottom rather than on the surface of sand columns.

Host-finding and penetration of *S. carpocapsae* were influenced by both the depth of the host and by their infection status (Fig. 2). Significantly more *S. carpocapsae* penetrated into the uninfected hosts than into the infected hosts. Penetration by *S. carpocapsae* was also greater when the hosts were placed on the surface rather than at the bottom of the sand columns. Nictation behavior of *S. carpocapsae* was enhanced by the presence of *S. glaseri* only when hosts were not present (Fig. 3).

Interaction on an agar surface: Host-finding behavior of *S. carpocapsae* on an agar surface was significantly increased ($P < 0.05$) by the presence of *S. glaseri* (Fig. 4). The percentage of *S. carpocapsae* IJ that found the caged hosts increased from 1.21 ± 0.69% to 9.35 ± 2.25% in the presence of *S. glaseri*. The host-finding of *S. glaseri* significantly decreased in the presence of *S. carpocapsae*. The percentage of *S. glaseri* IJ finding hosts was 39.41 ± 4.01% when applied alone but dropped to 21.33 ± 3.05% when applied together with *S. carpocapsae*.

Interaction in a sand column: Host-finding and penetration behavior of *S. carpocapsae* also was influenced by the presence of *S. glaseri* in the sand columns (Fig. 5). Significantly greater numbers ($P < 0.05$) of *S. carpocapsae* found and penetrated into hosts

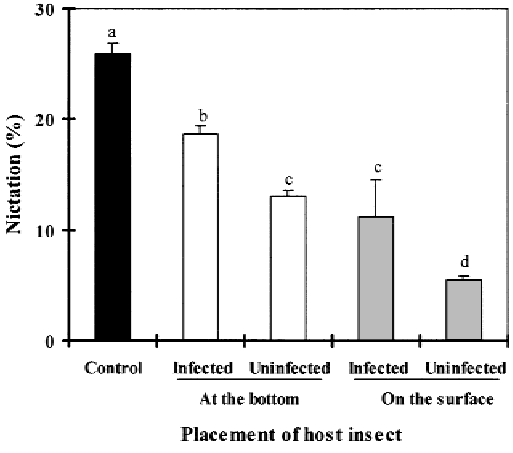

FIG. 1. Nictation rate of *Steinernema carpocapsae* infective juveniles (IJ) in the presence of infected or uninfected *Galleria mellonella* larvae placed at the bottom or on the surface of a sand column. One thousand *S. carpocapsae* IJ were added to the surface of the sand column. Small bars are standard errors of the means for five replicates. Bars with different letters differ significantly ($P < 0.05$).

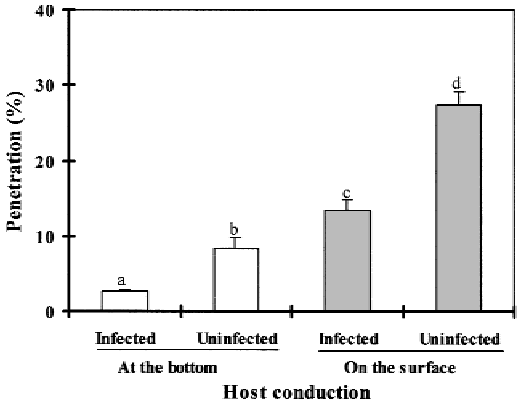

FIG. 2. Penetration rate of *Steinernema carpocapsae* infective juveniles (IJ) into infected or uninfected *Galleria mellonella* larvae placed at the bottom or on the surface of a sand column. One thousand *S. carpocapsae* IJ were added on the surface of the sand column. Small bars are standard errors of means for five replicates. Bars with different letters differ significantly ($P < 0.05$).

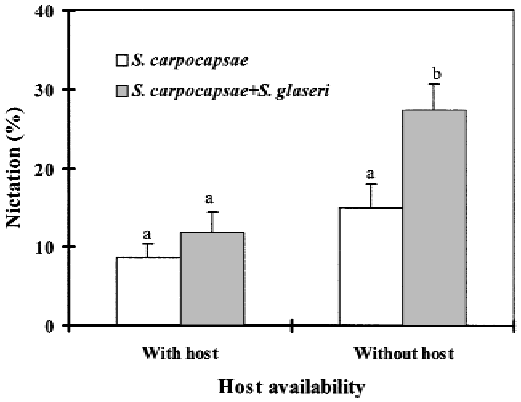

FIG. 3. Nictation rate of *Steinernema carpocapsae* infective juveniles (IJ) in the presence or absence of *S. glaseri* IJ with or without *Galleria mellonella* larvae at the bottom of a sand column. Small bars are standard errors of means for five replicates. Bars in a pair with different letters differ significantly ($P < 0.05$).

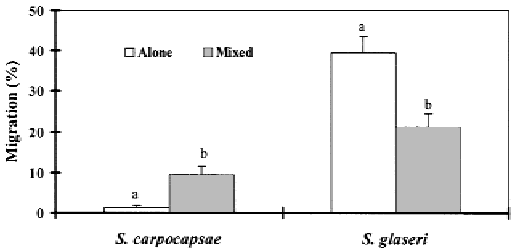

FIG. 4. Migration of *Steinernema carpocapsae* and *S. glaseri* infective juveniles (IJ) to *Galleria mellonella* larvae as affected by the presence of the other species. The rate was recorded 5 hours after application. Small bars are standard errors of means for five replicates. Bars in a pair with different letters differ significantly ($P < 0.05$).

placed at the 2-cm depth when they were applied with *S. glaseri* than when applied alone (Fig. 5A). A similar trend was observed when the hosts were placed at a depth of 7 cm (Fig. 5B). *Steinernema carpocapsae* out-competed *S. glaseri* when hosts were placed at the 2-cm depth, whereas *S. glaseri* out-competed *S. carpocapsae* at a depth of 7 cm. The sex ratio of *S. carpocapsae* that penetrated hosts at a depth of 2 cm in the presence of *S. glaseri* was also more male-biased than those that penetrated hosts in the absence of *S. glaseri* (Fig. 6).

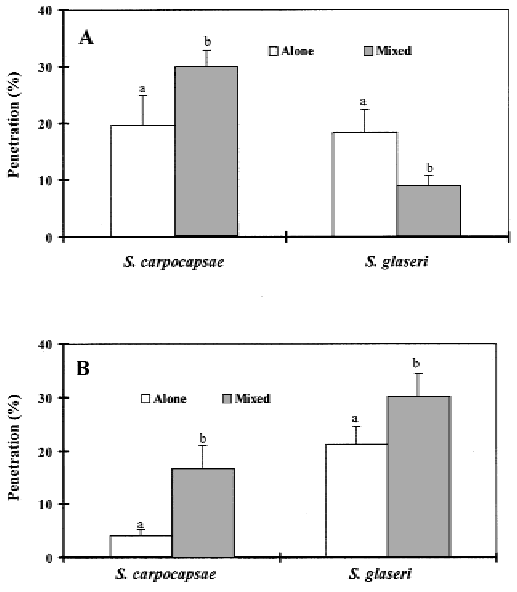

FIG. 5. Penetration of *Steinernema carpocapsae* or *S. glaseri* infective juveniles (IJ) into *Galleria mellonella* larvae placed at different depths. A) Placement 2 cm deep. B) Placement 7 cm deep. in a 7-cm sand column. One milliliter of water containing 1,000 *S. carpocapsae* or *S. glaseri* IJ alone or 1 ml with 500 IJ each of the two nematode species was added to the surface of the sand column. Small bars are standard errors of means for five replicates. Bars in a pair with different letters differ significantly ($P < 0.05$).

DISCUSSION

Infection behavior of entomopathogenic nematodes is characterized by their foraging strategies. Nictation behavior is an effective ambushing tactic employed by *S. carpocapsae* because it increases the surface area contacting a passing host and reduces the surface tension forces holding the nematode to the substrate (Campbell and Gaugler, 1993). Nictation behavior is not sustained at all times, as it is often followed by ''bridging'' and ''leaping'' related to active dispersal (Ishibashi and Kondo, 1990). The present study indicates that the nictation of *S. carpocapsae* IJ is largely influenced by the presence or the location of host insects. In the presence of a host insect, whether alive or dead, IJ migrate to the host and do not nictate as much at the inoculation point. The higher penetration rate of *S. carpocapsae* IJ when the host was directly exposed to nematodes (host on the surface of sand column) may have been due to their inclination to remain at the inoculation point. Penetration rates precipitously decreased when the host

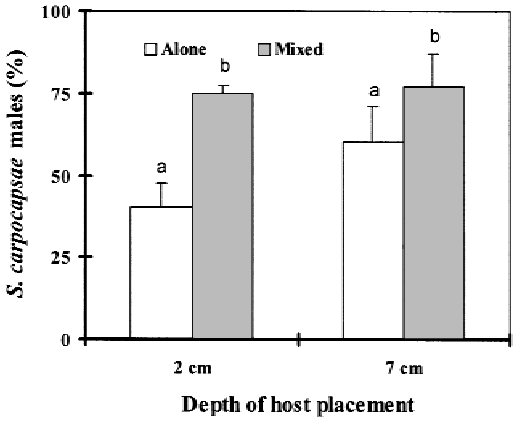

FIG. 6. Sex ratio (percent males) of *Steinernema carpocapsae* infective juveniles (IJ) penetrating into *Galleria mellonella* larvae placed at 2 cm or 7 cm below the surface in a 7-cm sand column after application of *S. carpocapsae* IJ alone or with *S. glaseri* IJ on the sand surface. Small bars are standard errors of means for five replicates. Bars in a pair with different letters differ significantly ($P < 0.05$).

was placed away from the inoculation point. In the present investigation, more *S. carpocapsae* IJ penetrated into an uninfected insect than into an infected insect, as was previously reported (Glazer 1997; Wang and Ishibashi, 1997), suggesting that subsequent IJ penetration is suppressed to some extent by substances emanating from the penetrated nematodes or from the symbiotic bacteria.

The present study also suggests that *S. carpocapsae* IJ are activated to move to the host insect by the presence of the competitor *S. glaseri,* whereas this coexistence negatively influences *S. glaseri.* This was an unexpected result because *S. glaseri* IJ move more actively than *S. carpocapsae* and usually move downward to locate a sedentary host in the soil. Penetration of hosts at the 2-cm depth by *S. glaseri* decreased in the mixed inoculation with *S. carpocapsae,* contrary to the increase in penetration by *S. carpocapsae.* However, *S. glaseri* had a higher penetration rate than *S. carpocapsae* in the deeper depth. *Steinernema carpocapsae* IJ are likely to be activated by the presence of competing nematodes, though they are inclined to become quiescent when alone. *Steinernema glaseri* IJ forage actively for hosts in the deeper depth, consistent with their categorization as cruisers. Therefore, *S. glaseri* was more dominant at the 7-cm depth than at the 2-cm depth. Our results are consistent with Koppenhofer et al. (1996), who reported that *S. glaseri* was superior to *S. carpocapsae* when hosts were located deeper in the soil.

The ratio of males among penetrating *S. carpocapsae* IJ increased with increasing distance to host insects (Grewal et al., 1993; Ishibashi et al., 1994). A high male ratio also occurred when *S. glaseri* was present. We suggest that male *S. carpocapsae* IJ may be more active in the presence of competitors. The results of the present study may be partly explained by the male colonization strategy used by *S. carpocapsae* for ensuring reproductive success.

Literature Cited

Campbell, J. F., and R. Gaugler. 1993. Nictation behavior and its ecological implications in entomopathogenic nematode search strategies. Behaviour 126:155–169.

Dunphy, G. B., T. A. Rutherford, and J. M. Webster. 1985. Growth and virulence of *Steinernema glaseri* influenced by different subspecies of *Xenorhabdus nematophilus.* Journal of Nematology 17:476–482.

Dutky, S. R., J. V. Thompson, and G. E. Cantwell. 1964. A technique for the mass propagation of the DD-136 nematode. Journal of Insect Pathology 6:417–422.

Georgis, R., and R. Gaugler. 1991. Predictability in biological control using entomopathogenic nematodes. Journal of Economic Entomology 84:713–720.

Glazer, I. 1997. Effects of infected insects on secondary invasion of steinernematid entomopathogenic nematodes. Parasitology 114:579–604.

Grewal, P. S., M. Matsuura, and V. Converse. 1997. Mechanisms of specificity of association between the nematode *Steinernema scapterisci* and its symbiotic bacterium. Parasitology 114:483–488.

Grewal, P. S., S. Selvan, E. E. Lewis, and R. Gaugler. 1993. Male insect-parasitic nematodes: A colonizing sex. Experientia 49:605–608.

Ishibashi, N., and E. Kondo. 1990. Behavior of infective juveniles. Pp. 139–150 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.

Ishibashi, N., X. D. Wang, and E. Kondo. 1994. *Steinernema carpocapsae:* Poststorage infectivity and sex ratio of invading infective juveniles. Japanese Journal of Nematology 24:60–68.

Kaya, H. K. 1990. Soil ecology. Pp. 93–117 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.

Kondo, E. 1989. Studies on the infectivity and propagation of entomogenous nematodes, *Steinernema* spp., to the larvae of the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae), on the soil surface. Applied Entomology and Zoology 21:553–560.

Koppenhofer, A. M., M. E. Baur, and H. K. Kaya. 1996. Competition between two steinernematid nematode species for an insect host at different soil depths. Journal of Parasitology 82:34–40.

Koppenhofer, A. M., H. K. Kaya, S. Shanmugan, and G. L. Wood. 1995. Interspecific competition between steinernematid nematodes within an insect host. Journal of Invertebrate Pathology 66:99–103.

Lewis, E. E., R. Gaugler, and R. Harrison. 1992. Entomopathogenic nematode host finding: Response to host contact cues by cruise and ambush foragers. Parasitology 105:103–107.

Miller, R. W. 1989. Novel pathogenicity assessment technique for *Steinernema* and *Heterorhabditis* entomopathogenic nematodes. Journal of Nematology 21:574 (Abstr.).

Poinar, G. O., Jr. 1990. Taxonomy and biology of Steinernematidae and Heterorhabditidae. Pp. 23–61 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.

Wang, X. D., and N. Ishibashi. 1997. Infection of the entomopathogenic nematodes (Steinernematidae) on the subsequent invasion of infective juveniles. Japanese Journal of Nematology 28:8–16.