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Susceptibility of Asian longhorned beetle, *Anoplophora glabripennis* (Motchulsky) (Coleoptera: Cerambycidae) to entomopathogenic nematodes

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Abstract

Isolates of *Steinernema feltiae* SN from France, *Steinernema glaseri* NJ from New Jersey, *Steinernema riobrave* TX from Texas, *Steinernema carpocapsae* Sal from Indiana, *S. carpocapsae* All from Georgia, and *Heterorhabditis marelata* IN from Indiana were screened for efficacy against laboratory colonies of Asian longhorned beetle, *Anoplophora*, *glabripennis* collected from Queens, New York and Chicago, Illinois. Two bioassays were used to screen nematode effects on beetle larvae; a filter paper assay using a 24-h exposure of nematode-to-target-insect, and a diet cup bioassay using a 72-h exposure of host larvae to infective juveniles applied to the larval bore hole made in the artificial diet in the cups. First- and third-stage larvae were susceptible to all isolates using a filter paper bioassay. *S. feltiae* and *S. carpocapsae* Sal were the most effective, causing 100% mortality. *S. feltiae* was more infectious than *S. carpocapsae* Sal against third, sixth, and seventh instars. *S. riobrave*, *S. glaseri*, and *H. marelata* were ineffective against the older instars. In the diet cup bioassay, *S. feltiae* and *S. carpocapsae* Sal killed 71–100% of mid-to late instar larvae, but the remaining isolates screened were ineffective. Nematode preconditioning to aqueous *A. glabripennis* frass extracts inhibited *S. carpocapsae* Sal infectivity but had no effect on nematode pathogenicity. *S. feltiae* juveniles were positively attracted to *A. glabripennis* frass extracts. Our results demonstrate the potential use of *S. feltiae* and *S. carpocapsae* isolates as control agents for *A. glabripennis*.
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1. Introduction

The introduction of the Asian longhorned beetle, *Anoplophora glabripennis* (Motchulsky), to North America poses a serious threat to the maple syrup and lumber industries (Becker, 1998). In addition to maple, the beetle attacks a number of important shade tree species including birch, poplars, horse chestnuts, and elms (Haack et al., 1997). Female adult beetles make niches in the bark of trees and lay a single egg into each niche. The eggs hatch in 10–15 days (Lingafelter and Hoebeke, 2002), and larvae bore through the cambium and hardwood, eventually killing the tree (Smith et al., 2001). Current control of the beetle requires that infested trees be destroyed. The cryptic behavior of the larval stages limits the efficacy of conventional control methods, increasing the need for alternative treatments.

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditdae have the ability to locate and kill insects in cryptic environments (Begley, 1990). EPNs can target insects beneath tree bark (Finney, 1977; Finney and Walker, 1979; Solter et al., 2001) and in larval chambers (Abbas et al., 2000; Solter et al., 2001), and are reported to infect a number of insect pests that are difficult to treat using insecticides,

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including leafminers (Hara et al., 1993), palm weevils (Abbas et al., 2000), and bark beetles (Finney, 1977; Finney and Walker, 1979). Furthermore, recent laboratory and field studies have demonstrated susceptibility of *A. glabripennis* to EPN attack (Li, 1988; Liu et al., 1992, 1998; Qin et al., 1988; Solter et al., 2001).

Solter et al. (2001) demonstrated that Indiana isolates of *Steinernema carpocapsae* (Weiser) Sal and *Heterorhabditis marelata* Liu and Berry IN were more pathogenic to *A. glabripennis* than *Heterorhabditis indica* Poinar, Karunakar, and David HOM-1 and *Heterorhabditis bacteriophora* Poinar Lewiston. An LD_{50} for second- and third-instar larvae exposed to *H. marelata* infective juveniles (IJs) was estimated to be 19 IJs, and 347 IJs for fourth and fifth instars, respectively. The authors also reported the migration of *H. marelata* into larval chambers via adult beetle oviposition sites on test logs. Liu et al. (1998) reported a 66.7% reduction in new *A. glabripennis* emergence holes after injection of a Beijing strain of *S. carpocapsae* into existing emergence holes. Infected *A. glabripennis* larvae were capable of supporting nematode development, producing in the range of 200,000–900,000 IJs per insect (Solter et al., 2001).

Our objectives for these experiments were to determine the effectiveness of a broader range of entomopathogenic nematodes on *A. glabripennis* and to assess nematode responses to host volatiles.

2. Materials and methods

2.1. Source and maintenance of nematodes and insects

Isolates of *Steinernema feltiae* SN from France, *Steinernema glaseri* (Steiner) NJ from New Jersey, *Steinernema riobrave* Cabanillas, Poinar, and Raulston TX from Texas, *S. carpocapsae* isolates Sal from Indiana, *S. carpocapsae* All from Georgia, and *H. marelata* IN from Indiana were separately cultured at 25 °C in last instar *Galleria mellonella* (L.). IJs were harvested in White traps and washed by sedimentation in three changes of tap water (Kaya and Stock, 1997). Harvested IJs were stored at 15 °C and used within 3 weeks of emergence.

Laboratory colonies of *A. glabripennis* were established from larvae collected from Queens, New York and Chicago, Illinois, and shipped inside cut logs via truck to the Ansonia, Connecticut USDA Forest Service quarantine unit under a valid USDA-APHIS permit. The insects were reared in individual cups at 20 °C using a modified red oak borer diet (Galford, 1985). Relative humidity (RH) was maintained at 100% for the first 2 weeks of development before reducing RH to 60%. Larvae were transferred to new diet media every 2 weeks and to larger diet cups as they developed. Diet cup sizes were 59 ml for third instars, 118 ml for fifth instars, and 237 ml for seventh instars. All tests were conducted at the Ansonia quarantine facility and dead insects were shipped to the Illinois Natural History Survey for dissection and confirmation of nematode infection.

2.2. Effect of A. glabripennis frass extracts on nematode attraction

Three stacked 5-mm filter paper discs were placed 10 mm from the edge of a 90-mm petri dish filled with 1.5% bactoagar. A 37-μl aqueous frass extract suspension made from homogenized wood frass and water at a ratio of 10:1 water:frass (by weight), or water in the absence of frass, was added to the stacked discs. The dishes were arranged in a randomized complete block design and incubated for 2 h at 24 °C. Three stacked filter discs were placed in the center of the dish, 35 mm from the treatments, and 500 IJs in 20 μl water were added to these discs. Each of the following isolates, *S. feltiae*, *S. riobrave*, *S. carpocapsae* All, *S. carpocapsae* Sal, *S. glaseri*, and *H. marelata* were tested for attraction to *A. glabripennis* frass. Each experiment compared three of the six nematode treatments and each treatment was replicated 5 times. The dishes were sealed with Parafilm and incubated for 2 h at 24 °C. The volatile treated-filter discs and, separately, a 10-mm radius disc of agar circumscribing the discs were removed and left to soak overnight in water to remove any nematodes present. The number of nematodes attracted to the frass- and water-treated filter discs and those present on the agar discs were counted in the wash water. Three trials of each experiment were conducted. Each trial was treated as a replicate and the treatment means per experiment were analyzed using contrast analysis (SAS Institute, 1999).

2.3. Effect of entomopathogenic nematodes on A. glabripennis first instars

Two 15-mm filter papers (Whatman No. 1) were placed into test wells of four 24-well tissue culture plates (Corning Costar No. 3526, Cambridge, MA) laid side by side in a 2×2 matrix in a random block design. Ten first-instar *A. glabripennis* larvae were placed into each well and 100 IJs in 100 μl of water were added to each well. Six treatments were applied, *S. feltiae*, *S. carpocapsae* Sal, *S. glaseri*, *S. riobrave*, *H. marelata*, and water in the absence of nematodes, one treatment per well, to a total of seven wells per treatment. Two pieces of moistened tissue were placed into the lid of the plate to maintain a high humidity for first instars and nematodes. Plates were incubated for 24 h at 20 °C. Insect mortality was monitored daily for 3 days. The total number of live and dead larvae was counted for each treatment, not mortality for individual replicates.

Comparisons between nematode treatments and the control were conducted using χ^2 analysis.

2.4. Effect of entomopathogenic nematodes on A. glabripennis early and late instars

Third-, sixth-, and seventh-instar *A. glabripennis* larvae were exposed to *S. feltiae*, *S. carpocapsae* Sal, *S. glaseri*, *S. riobrave*, *H. marelata*, and a water control. A randomized complete block design of filter paper and diet cup bioassays were conducted to compare nematode treatments. All treatments in this and the remaining experiments were incubated at 20 °C. Unless otherwise stated, seven larvae were used for each treatment. Insect mortality was monitored for 2 weeks; host insects were dissected 3 days after death following incubation at 24 °C to assess nematode invasion. Insects killed from 4 to 14 days after infection were sent from the Ansonia quarantine facility to the Illinois Natural History Survey in several shipments for incubation and dissection. Total insect mortality was compared using Fisher Exact analysis. The number of nematodes per insect was compared between treatments using ANOVA (SAS Institute, 1999).

2.5. Filter paper bioassay

Larvae were placed individually into 60-mm petri dishes (Fisher Scientific, IL) lined with two pieces of filter paper. One hundred IJs in 1 ml water were added to the filter paper and the dishes were incubated for 24 h. The insects were removed, washed and dried, then transferred to clean 60-mm dishes for further incubation. In addition to *A. glabripennis* larvae, 16 adult beetles of various ages post-eclosion were exposed to 100 *S. carpocapsae* Sal and *S. feltiae* IJs, eight beetles per isolate.

2.6. Diet cup bioassay

A 100-μl aliquot of 100 IJs was applied to the larval bore hole in cups containing larvae and nutritional media, one larva per cup, and the cups were incubated for 72 h. The nematode-exposed larvae were removed, washed, blotted dry, and transferred to 60-mm petri plates for further incubation.

2.7. Effect of preconditioning on nematode infectivity

Three nematode isolates, *S. feltiae*, *S. carpocapsae* Sal, and *H. marelata* were exposed to 25 ml of *A. glabripennis* frass extracts, or a water control in a 50-ml centrifuge tube (Sarsedt), for 1 h prior to use. Filter paper and diet cup bioassays, as previously described, were conducted using *A. glabripennis* fourth, sixth, and eighth instars exposed to one of the six nematode treatments and a water treatment in the absence of nematodes. Seven larvae were used per treatment. Inoculated larvae were incubated for 72 h before dead insects were removed, washed, and sent to the Illinois Natural History Survey for dissection and nematode counts. There was no statistical difference between host instars for the preconditioning treatments; therefore the data were combined for each nematode species and each instar was treated as a replicate for probit analysis.

2.8. Effect of Steinernema carpocapsae Sal concentration on infectivity to A. glabripennis larvae

In an earlier trial at the Ansonia unit, six concentrations of *S. carpocapsae* Sal were tested in two experiments using a randomized complete block design against young (first and second instars) and mid-stage larvae (fourth and fifth instars). Nematodes were applied at 0, 10, 20, 30, 40, and 50 IJs per insect in a filter paper bioassay of young larvae, and 0, 50, 100, 200, 400, and 800 IJs per insect in a filter paper assay using mid-stage larvae. More insects were available for this experiment and each treatment contained 18 insects. Experiments were incubated at 20 °C for 72 h, then dead insects were removed, washed, and sent to the Illinois Natural History Survey. Dead larvae were placed in White traps to collect emerging nematodes.

2.9. Experimental constraints

The experiments we conducted were restricted in several ways, primarily due to the quarantine status of *A. glabripennis* and issues involving the availability of larvae for testing. The beetles are fastidious, have a long development time, are highly labor-intensive to rear, and must be reared in a regulation Biological Level 3 quarantine unit. The numbers of larvae available for these experiments were therefore limited, which in turn limited options for testing a variety of nematode species. Additionally, we were constrained to a choice of one temperature for testing of the nematodes against host larvae, both because rearing chambers at the Ansonia, Connecticut quarantine unit were set at the optimal rearing temperature for *A. glabripennis* (20 °C) and because the low numbers of available larvae precluded a broader testing scheme.

Both insect pathologists involved in this study were housed at the Illinois Natural History Survey, necessitating shipment of the inoculated larvae between cooperating institutions. The quarantine regulations for *A. glabripennis* required that larvae be dead before they could be shipped, a situation that lengthened the time period slightly before the larvae could be examined.

Because the experiments of Solter et al. (2001) were constrained for the same reasons, we elected to

re-examine their preliminary results, essentially providing more trials for the nematode species that were successfully tested in that study.

All experiments were conducted using a randomized complete block design. The Fisher exact test was used to compare number of dead larvae between nematode treatments and a water control in the absence of nematodes. ANOVA (SAS Institute, 1999) was used to compare numbers of penetrated nematodes between the isolates in the infectivity bioassays. Probit analysis (SAS Institute, 1999) was used to calculate LC_{50} and LT_{50} values over a range of nematode concentrations.

3. Results

3.1. Effect of A. glabripennis volatiles on nematode attraction

In the first series of experiments, greater numbers of *S. feltiae* SN IJs were recovered from the filter discs than were *S. carpocapsae* All and *S. riobrave* TX ($F = 4.65$; $df = 2, 15$; $P = 0.037$). A significant interaction between attractant and nematode isolates was observed ($F = 7.34$; $df = 2, 15$; $P = 0.011$); *S. feltiae* aggregated on the *A. glabripennis* frass-extract filter discs ($F = 3.64$; $df = 5, 12$; $P = 0.038$; Fig. 1A′). There was no effect of frass extracts on *S. riobrave* and *S. carpocapsae* All. In the second series of comparisons, there was no aggregation by *S. feltiae* on *A. glabripennis* frass-extract filter discs (Fig. 1B′). Greater numbers of *H. marelata* IN IJs were collected from the filter discs (7.1 ± 1.8) than *S. carpocapsae* All (2.9 ± 0.6) and *S. feltiae* (3.4 ± 0.7) ($F = 7.58$; $df = 2, 15$; $P = 0.010$). Greater numbers of *H. marelata* IJs were recovered at the *A. glabripennis* frass-extract treated discs (10.2 ± 2.6) than at control discs (4.1 ± 1.0), but the interaction between nematodes and extracts was insignificant ($P > 0.05$; Fig. 1B′). In the final set of tests, *S. glaseri* NJ showed greater preference to *A. glabripennis* frass-extract discs than to control disks, but the effect was highly variable among replicates ($P > 0.05$). In all three tests where nematode numbers were higher on the treated filter disks, no increase of nematode numbers was recorded from the 10-mm radius around the treated filter discs.

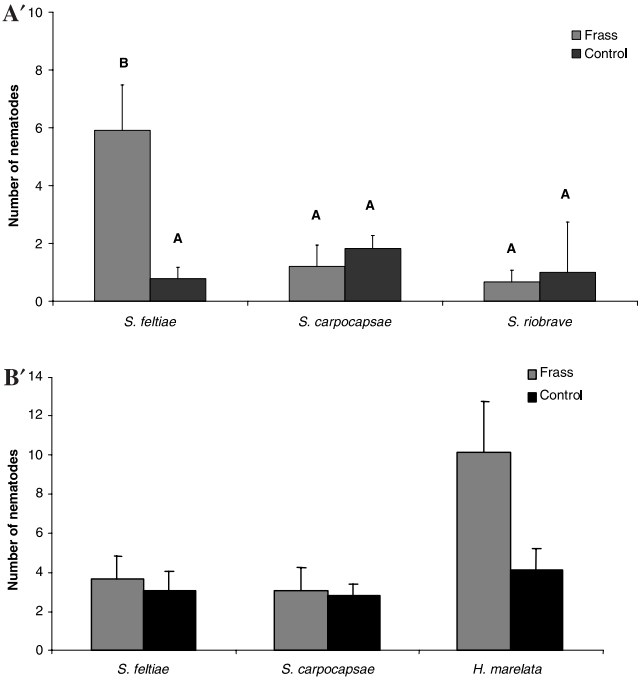

Fig. 1. Number (±SEM) of *S. feltiae* SN, *S. carpocapsae* All, and *S. riobrave* TX IJs (A′), *S. feltiae* SN, *S. carpocapsae* Sal, and *H. marelata* IN (B′) attracted to *A. glabripennis* frass-extract filter discs, and sterile, distilled water on 1.5% bacto-agar plates after 2 h. Bars headed with the same letter are not different between treatments according to contrast analysis ($P \leqslant 0.05$) ($F = 3.64$; $df = 5, 12$; $P = 0.038$; (A), $P > 0.05$; (B)).

3.2. Effect of entomopathogenic nematodes on A. glabripennis first instars

First-instar *A. glabripennis* larvae were susceptible to all isolates screened. *S. feltiae* caused the highest neonate mortality at 97% ($P < 0.0001$; Fig. 2) and *H. marelata* the lowest at 39% ($P = 0.009$; Fig. 2). First instar mortality in the water treatments was 19%. In general, the steinernematids were more pathogenic to first-instar larvae than was *H. marelata*.

3.3. Effect of entomopathogenic nematodes on A. glabripennis early and late instars

Steinernema feltiae and *S. carpocapsae* Sal were the most pathogenic isolates against third-, sixth-, and

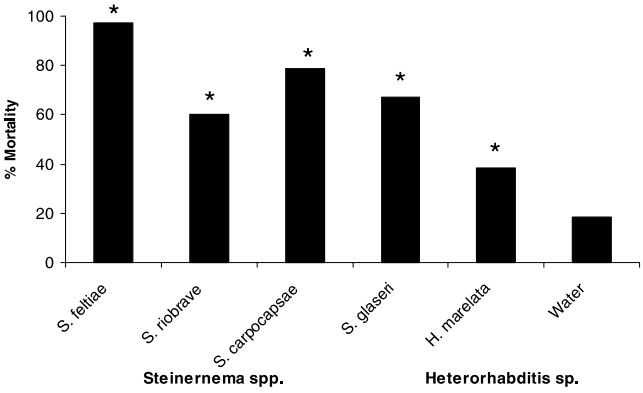

Fig. 2. Mortality of *A. glabripennis* first instars exposed to 100 *S. feltiae* SN, *S. riobrave* TX, *S. carpocapsae* Sal, *S. glaseri* NJ, *H. marelata* IN IJs or water after 24-h exposure on 10-mm filter discs in 24-well tissue culture plates. Bars headed by an "*" are significantly different from the control by χ^2 comparison of killed larvae.

seventh-instar host larvae in the filter paper and diet cup bioassays ($P < 0.01$; Figs. 3–5A and B). Mortality caused by *S. glaseri* and *S. riobrave* was significantly lower and more variable (Figs. 3,4,5A). In the diet cup bioassays, *S. riobrave*, *S. glaseri*, and *H. marelata* had no effect on larval mortality. In the filter paper assays, infectivity, as measured by numbers of recovered nematode adults, was greater in *S. feltiae* than *S. carpocapsae* Sal ($F = 48.29$; $df = 4, 41$; $P = 0.0001$; Fig. 3A, $F = 111.09$; $df = 4, 41$; $P < 0.0001$; Fig. 4A, $F = 14.36$; $df = 4, 41$; $P < 0.0001$; Fig. 5A), and each were more infectious than *S. riobrave*, *S. glaseri*, and *H. marelata* ($P < 0.05$). In the diet cup bioassays, *S. feltiae* and *S. carpocapsae* Sal were equally infective to third instars ($F = 131.89$; $df = 4, 41$; $P < 0.0001$; Fig. 3B), but infectivity dropped in sixth and seventh instars to less than two nematodes recovered per insect ($P > 0.05$) for both species.

3.4. Effect of preconditioning nematode on infectivity

Nematode preconditioning to *A. glabripennis* frass extracts adversely affected *S. carpocapsae* Sal infectivity ($F = 13.37$; $df = 3, 11$; $P = 0.0062$); numbers of penetrating IJs dropped from 4.3 ± 1.2 following water treatment to 1.7 ± 0.7 after *A. glabripennis* frass-extract exposure. There was no effect of frass extract on *S. feltiae* infectivity. Frass extracts had no effect on pathogenicity of *S. feltiae* and *S. carpocapsae* Sal ($P > 0.05$).

3.5. Effect of S. carpocapsae Sal concentration on infectivity to A. glabripennis

The LD_{50} for second/third and fourth/fifth instar *A. glabripennis* larvae exposed to *S. carpocapsae* Sal was estimated at 9 IJs (95% CI 4–12 IJs; $\chi^2 = 12.14$; $P = 0.005$) and 17 IJs (95% CI 0.01–42 IJs; $\chi^2 = 4.33$; $P = 0.0374$), respectively. Nematode yields were greatest

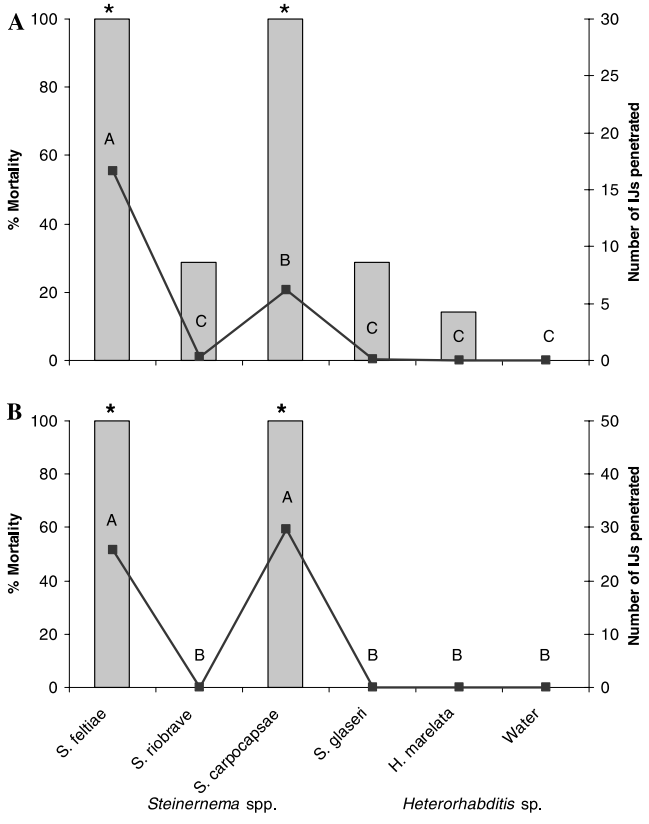

Fig. 3. Mortality (bar) and nematode infectivity (line) of third-instar *A. glabripennis* larvae exposed to *S. feltiae* SN, *S. riobrave* TX, *S. carpocapsae* Sal, *S. glaseri* NJ, *H. marelata* IN, and water after 24-h exposure on filter paper (A), and 72-h exposure in larval diet cups at 20 °C (B). Single larvae were each exposed to 100 IJs ($N = 7$ larvae/treatment). Log transformed adult counts; line points headed with the same letter are not different among treatments according to Duncan's multiple range test ($P \leqslant 0.05$) ($F = 48.29$; $df = 4, 41$; $P < 0.0001$; (A), $F = 131.89$; $df = 4, 41$; $P < 0.0001$; (B)). Bars headed by an "$*$" are significantly different from the control by Fisher Exact test comparison of killed larvae.

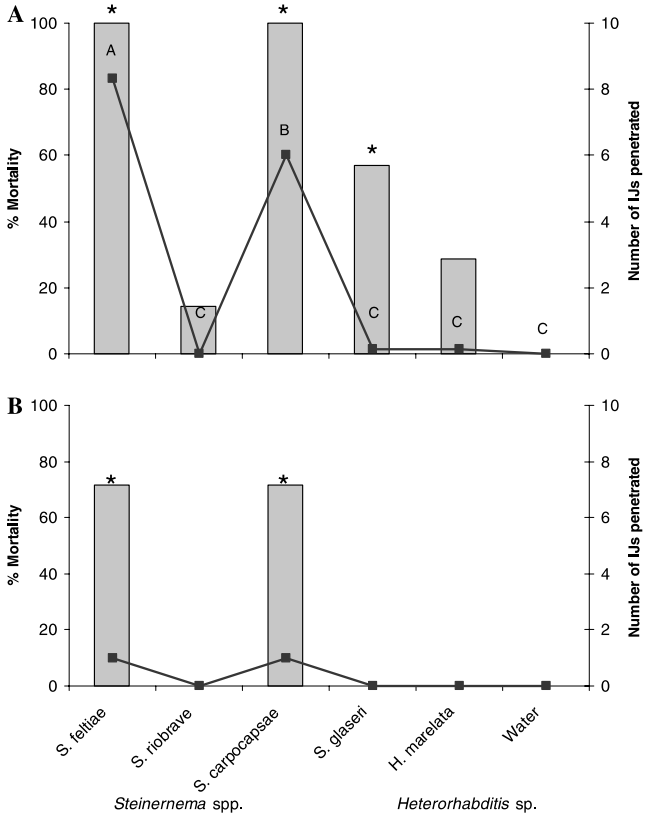

Fig. 4. Mortality (bar) and nematode infectivity (line) of *A. glabripennis* sixth-instar larvae exposed to *S. feltiae* SN, *S. riobrave* TX, *S. carpocapsae* Sal, *S. glaseri* NJ, *H. marelata* IN, and water after 24-h exposure on filter paper (A), and 72-h exposure in larval diet cups at 20 °C (B). A single larva was exposed to 100 IJs ($N = 7$ larvae/treatment). Log transformed adult counts; line points headed with the same letter are not different among treatments according to Duncan's multiple range test ($P \leqslant 0.05$) ($F = 111.09$; $df = 4, 41$; $P < 0.0001$; (A), not significant; (B)). Bars headed by an "$*$" are significantly different from the control by Fisher Exact test comparison of killed larvae.

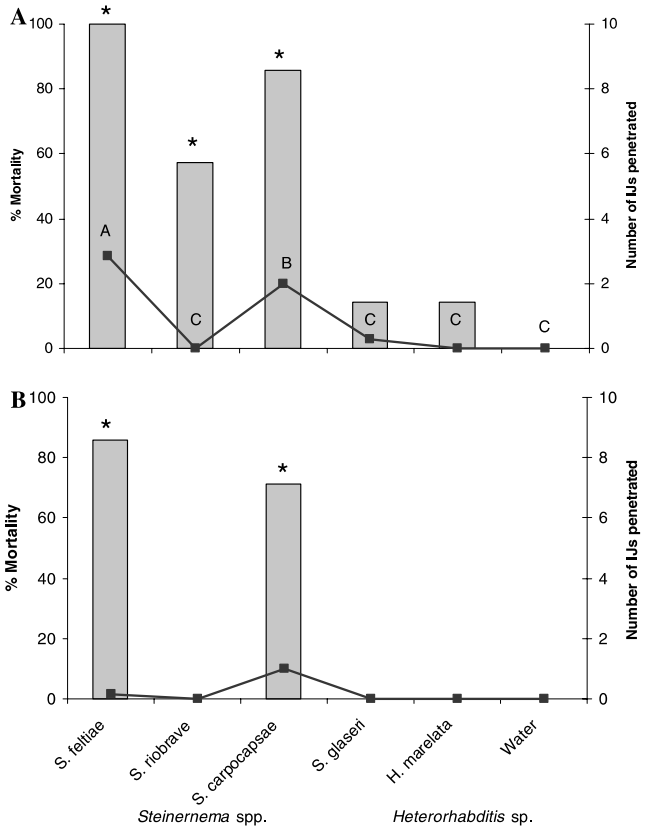

Fig. 5. Mortality (bar) and nematode infectivity (line) of *A. glabripennis* seventh-instar larvae exposed to *S. feltiae* SN, *S. riobrave* TX, *S. carpocapsae* Sal, *S. glaseri* NJ, *H. marelata* IN, and water after 24-h exposure on filter paper (A), and 72-h exposure in larval diet cups at 20 °C (B). A single larva was exposed to 100 IJs ($N = 7$ larvae/treatment). Log transformed adult counts; line points headed with the same letter are not different among treatments according to Duncan's multiple range test ($P \leqslant 0.05$) ($F = 14.36$; $df = 4, 41$; $P < 0.0001$; (A), not significant; (B)). Bars headed by an "$*$" are significantly different from the control by Fisher Exact test comparison of killed larvae.

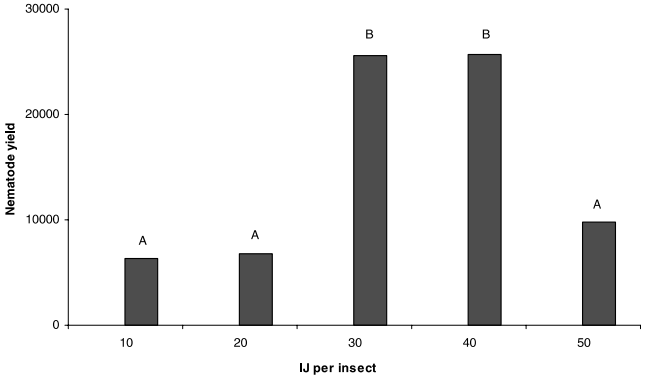

Fig. 6. *Steinernema carpocapsae* Sal yields in *A. glabripennis* second- and third-instar larvae exposed to six concentrations of infective juveniles (IJ) (10–50) on filter paper. $N = 18$ larvae/treatment. Log transformed yields; bars headed with the same letter are not different among treatments according to Duncan's multiple range test ($P \leqslant 0.05$) ($F = 3.42$; $df = 4, 95$; $P = 0.0334$).

at concentrations of 30 and 40 IJs per insect compared to 10, 20, and 50 nematodes per insect ($F = 3.42$; $df = 4, 95$; $P = 0.0334$; Fig. 6) but were unaffected at nematode concentrations of 100–800 ($P > 0.05$). However, in the latter case, the lowest yields were observed at the highest concentration.

4. Discussion

Steinernema feltiae SN and *S. carpocapsae* Sal were the most effective of six nematode isolates tested against *A. glabripennis* larvae. Host larvae were more susceptible to infection by *S. feltiae* than *S. carpocapsae* Sal in filter paper bioassays, but were equally susceptible to the two isolates in diet cup bioassays. Infectivity as measure of EPN efficacy is subjective. Kondo and Ishibashi (1986) demonstrated that invasion by different nematode species varied quantitatively; a higher number of nematodes penetrating caused higher insect mortality. Dunphy and Webster (1986) suggested that differences in efficacy between two strains of *S. carpocapsae* were the result of differences in nematode infectivity. However, *Heterorhabditis* spp. often infect at lower rates than *Steinernema* spp. (Gouge et al., 1999; Menti et al., 2000), but comparative mortality is often similar or higher (Saunders and Webster, 1999; Shapiro et al., 1999, 2002). The discrepancy in infectivity between heterorhabditids and steinernematids may be attributed to differences in the time of establishment of the symbiotic bacteria in the insect host. Saunders and Webster (1999) noted the time of insect death by *H. megidis* Poinar, Jackson, and Klein was associated with the presence of significant numbers of *Photorhabdus*. In our current study, the low infectivity and insect mortality by *H. marelata* IN may have been attributed to the failure of its symbiont to establish in *A. glabripennis*. However, Solter et al. (2001) did demonstrate efficacy of *H. marelata* against *A. glabripennis*. This may be attributed to the longer exposure period used by Solter et al. (2001) in their bioassays, and the greater length of time required to penetrate or kill *A. glabripennis* by this heterorhabditid; 3–7 days compared to 2–3 days for steinernematids. The 24-h exposure period used in our study may have been insufficient for heterorhabditids to penetrate the host, accounting, in part, for the low mortality by *H. marelata*.

The use of the traditional filter-paper nematode bioassay demonstrated a broad susceptibility of *A. glabripennis* larvae to nematode infection, but the diet cup bioassays demonstrated particularly high larval susceptibility to *S. feltiae* and *S. carpocapsae* Sal. The latter test better mimics the ecological environment of *A. glabripennis* for host seeking by nematodes. Larval resistance to nematode infection increased as the larvae matured. *S. feltiae* was strongly attracted to *A.*

glabripennis volatiles, but *S. carpocapsae* Sal was negatively affected by exposure to the frass extracts, causing suppression of infectivity. Yields of *S. carpocapsae* Sal in second and third instars were low relative to yields in third and fourth instars as demonstrated by Solter et al. (2001), as smaller larvae support fewer nematodes.

Steinernema carpocapsae isolates are infective to a range of coleopteran wood boring species including *A. glabripennis* (Solter et al., 2001) and *Scolytus scolytus* (Fabricius) (Finney, 1977; Finney and Walker, 1979). *S. carpocapsae* adopt a sit-and-wait ambusher strategy (Lewis et al., 1992a,b), which is ideally suited to the protective environment of wood-borer chambers. However, *S. carpocapsae* can be ineffective against wood boring pests in the field (Finney, 1977; Finney and Walker, 1979) requiring direct injection into pest galleys to be successful (Lindegren and Barnett, 1982; Lindegren et al., 1981; Saleh and Abbas, 1998). *S. carpocapsae* was not responsive to *A. glabripennis* frass volatiles, and infectivity was reduced by exposure to *A. glabripennis* volatiles. Lewis et al. (1992a,b) reported no response of *S. carpocapsae* to *Spodoptera exigua* (Hübner) and *Popillia japonica* Newman frass and host cuticle, and to volatiles associated with *G. mellonella*. Similarly, Grewal et al. (1993) reported no increase in head thrusting, a behavior presumed to be associated with host-penetration, by *S. carpocapsae* exposed to gut contents of *Acheta domesticus* (L.), *Blatella germanica* (L.), *P. japonica*, and *S. exigua.* Grewal et al. (1993) suggested *S. carpocapsae* may respond in vivo to different factors such as hemolymph, or are unresponsive to gut contents if the nematode uses alternative penetration sites such as spiracles. Suppression of infectivity could occur in chambers containing large quantities of frass and may account for the variable effects of *S. carpocapsae* reported by Finney (1977) and Finney and Walker (1979) against another wood-boring species, *S. scolytus*, in the field. Toxicity of frass components to nematodes was not examined, so it is unclear if inhibition of infectivity was a factor of beetle volatiles or a component of the digested wood.

Steinernema feltiae exhibits both an active cruiser and ambusher strategy (Gaugler et al., 1994; Ishibashi and Kondo, 1990), and was partially responsive to *A. glabripennis* volatiles. *S. feltiae* was more infectious than *S. carpocapsae* to *A. glabripennis* in the filter paper bioassays. The ability of *S. feltiae* to utilize two host-seeking strategies and respond to host volatiles provides greater opportunity for infection than the more passive host-seeking *S. carpocapsae*. However, in the diet cup bioassays, there was no difference in nematode infectivity between the two species, suggesting that both strategies were important in the location of *A. glabripennis*. It is uncertain how this may correlate to a field situation because tunnels bored by larvae in artificial diet within the limited area of a diet cup would be easier to navigate than larger tunnels bored in hard wood in the beetle's natural environment.

Bioassay temperature may have favored *S. feltiae* isolates over the other isolates tested. Penetration efficacy of *S. feltiae* in *G. mellonella* was greater than 25 nematodes per insect at temperatures between 8 and 28 °C, with optimum IJ emergence at 15 and 20 °C (Hazir et al., 2001). Maximum nematode penetration by *S. riobrave* and *S. carpocapsae* in a lepidopteran host, *Pectinophora gossypiella* (Saunders), tested at temperatures between 9.9 and 40.0 °C was 28.5 and 24.9 °C, respectively, with greater than 20 nematodes per host (Gouge et al., 1999). However, at 20.0 °C the number of nematodes per host for both *S. riobrave* and *S. carpocapsae* dropped to less than 5 (Gouge et al., 1999). A study by Shapiro et al. (2002) on susceptibility of *Conotrachelus nenuphar* (Hebst) to EPNs at 25 °C demonstrated significant reduction in live larvae by *S. feltiae* and *S. riobrave*, but little effect by *S. carpocapsae* and *H. marelata.* At the cooler temperatures used in this study, the efficacy of *S. riobrave* and *S. carpocapsae* may have been reduced. *S. glaseri* KG can survive and remain infective in moist sand at temperatures of 15–28 °C for over 12 weeks (Molyneux, 1985), so was likely unaffected by the bioassay temperature. The impact of temperature on field released EPNs would be significant as seasonal conditions change. Further studies on temperature effects would be appropriate in this regard.

Susceptibility of *A. glabripennis* to nematode penetration decreased as larvae matured; first instars were the most susceptible stage and seventh instar the least. A sturdier cuticle and well-developed sieve plates may restrict nematode penetration in older larvae. Steinernematids penetrate their hosts through natural openings (Poinar, 1990) and Grewal et al. (1993) suggested that spiracle penetration was an important entry route for *S. carpocapsae* in certain hosts. It is unlikely that a more robust cuticle would limit steinernematid entry into older larvae. The high mortality of nematode penetrated larvae, including later instars penetrated by fewer nematodes, suggests little natural resistance to *Xenorhabdus* spp., the symbiotic bacteria associated with steinernematids. Adult beetles required a minimum of 7 days to die in filter paper bioassays.

Additional bioassays using *A. glabripennis* cohorts from different populations are needed to confirm the effects of EPNs on different life stages and further assess the potential for use of EPNs under field conditions. The presence of saprophytic nematodes in dead *A. glabripennis* larvae removed from infested trees (L. Bauer, personal communication) and records of field studies using nematodes against *A. glabripennis* in China (Li, 1988; Liu et al., 1992, 1998; Qin et al., 1988), however, suggest that the environment in larval galleries may be suitable for EPN survival and host seeking. Entomo-

pathogenic nematodes are a potential suppressive agent for *A. glabripennis*, possibly applied by flooding oviposition sites or emergence holes, and *Steinernema* species, particularly *S. feltiae*, may be suitable due to attraction to the host, host seeking capability, a relatively low lethal time, and activity at lower temperatures.

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