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Steinernema scarabaei for the control of white grubs

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Abstract

The efficacy of the new entomopathogenic nematode species, *Steinernema scarabaei*, isolated from white grubs in New Jersey for the control of economically important white grub species (Coleoptera: Scarabaeidae) was compared to that of two strains of *Steinernema glaseri*, four strains/isolates of *Heterorhabditis bacteriophora* (including two fresh isolates from scarab larvae), and an undescribed *Heterorhabditis* species from Korea. The efficacy was tested against the oriental beetle, *Exomala* (= *Anomala*) *orientalis*, the Japanese beetle, *Popillia japonica*, and the northern masked chafer, *Cyclocephala borealis*, under laboratory, greenhouse, and field conditions, and against the European chafer, *Rhizotrogus majalis*, in the laboratory. Under laboratory and greenhouse conditions, *S. scarabaei* was highly pathogenic to *P. japonica*, *E. orientalis*, and *R. majalis*, but was less effective against *C. borealis*. However, *S. scarabaei* provided excellent control of *P. japonica*, *E. orientalis*, and *C. borealis* under field conditions. *P. japonica* was the most nematode-susceptible white grub species. Against this species the superiority of *S. scarabaei* over the other nematodes tested became only apparent under field conditions and with very low nematode rates in the laboratory. *C. borealis* was less susceptible to all nematodes tested and only in a field experiment did *S. scarabaei* clearly outperform *H. bacteriophora*. Both *E. orientalis* and *R. majalis* were highly susceptible to *S. scarabaei* but showed moderate to low susceptibility to all other nematodes tested. In a field experiment, *S. scarabaei* also controlled *P. japonica* and *E. orientalis* larvae more quickly than *H. bacteriophora*. There was weak synergism between *S. scarabaei* and the neonicotinoid insecticide imidacloprid against *E. orientalis* larvae but not against *P. japonica* and *C. borealis* larvae. Overall, *S. scarabaei* shows exceptional potential for the biological control of white grubs.

Keywords: *Exomala orientalis*; *Popillia japonica*; *Cyclocephala borealis*; *Rhizotrogus majalis*; *Heterorhabditis*; *Steinernema*; Scarab; Insect–parasitic nematodes; Biological control; Integrated pest management

1. Introduction

White grubs, the root-feeding larvae of scarab beetles (Coleoptera: Scarabaeidae), cause significant damage to many agricultural and horticultural plants. In the United States, larvae of the introduced Japanese beetle, *Popillia japonica* Newman, are a major pest of turfgrass and ornamentals throughout much of the eastern states, and native masked chafers, *Cyclocephala* spp., larvae are major turfgrass pests and pests in ornamentals throughout the Midwest and western states (Potter, 1998; Vittum et al., 1999). In the Northeast and along the eastern seaboard, the European chafer, *Rhizotrogus majalis* (Razoumowsky), the Asiatic garden beetle, *Maladera castanea* (Arrow), and especially the oriental beetle, *Exomala* (= *Anomala*) *orientalis* Waterhouse have become similar in importance as turfgrass and ornamental pests as the Japanese beetle (Alm et al., 1999; Koppenhöfer, unpublished data). All these white grub species have an annual life cycle with adults emerging in summer to lay eggs in the soil among the roots of the host plants of the larvae (Potter, 1998; Vittum et al., 1999). By late summer most larvae have developed into the third instar. After overwintering the larvae resume feeding in spring until pupation in late spring. The extensive feeding activity of the larger larvae can kill large areas of grass especially under warm dry conditions (Potter, 1998; Vittum et al., 1999). In addition, vertebrate predators can tear up the turf to feed on the grubs even at relatively low larval densities (Potter, 1998; Vittum et al., 1999).

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Presently, chemical insecticides are still the first choice for turfgrass managers in the management of white grub pests. However, the implementation of the Food Quality Protection Act of 1996 (FQPA) (Anonymous, 1996) has already led and will continue to lead to the loss of many insecticides for curative white grub control in turfgrass. The widely used neonicotinoid imidacloprid and the molt accelerating compound halofenozide are less hazardous than organophosphates and carbamates and have small direct effect on beneficial invertebrates (Kunkel et al., 1999, 2001) but they are effective only when used preventively targeted at the difficult to detect eggs and young larvae. This results in the treatment of large areas that otherwise would have needed only partial or no treatment. Preventive applications of these compounds are very expensive, increase the chances of resistance development, and may have unintended environmental consequences. In the long-term their high efficacy against many turfgrass pests combined with their large-area applications is likely to reduce predators, parasitoids and pathogens of white grubs and other insect pests by depriving them of prey/hosts. Ultimately, this approach may increase dependency on chemical control. In addition, halofenozide provides only limited control of *E. orientalis* and *R. majalis* and no control of *M. castanea* larvae (Cowles et al., 1999; Koppenhöfer and Fuzy, in press). *M. castanea* is also resistant to imidacloprid (Koppenhöfer and Fuzy, in press; Vittum and Luce, 2002).

Entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) offer an environmentally safe and IPM compatible alternative to chemical insecticides for the control of white grubs. When applied under conducive conditions these nematodes have been as effective as chemical insecticides against *P. japonica* larvae (Georgis and Gaugler, 1991). However, *Cyclocephala* spp. larvae appear to be less susceptible to entomopathogenic nematodes (Koppenhöfer et al., 2000a,c; Shapiro-Ilan et al., 2002). Information on the nematode-susceptibility of other white grub species is scarce, but species such as *E. orientalis*, *R. majalis*, or *M. castanea* appear to be less susceptible to the commonly used nematode species and strains such as *Heterorhabditis bacteriophora* Poinar and *Steinernema glaseri* (Steiner) (Koppenhöfer, unpublished data; Koppenhöfer et al., 2002; Simard et al., 2001).

We have recently isolated a new nematode species, *Steinernema scarabaei* Stock and Koppenhöfer (Stock and Koppenhöfer, 2003) from epizootics in larval populations of *E. orientalis* and *P. japonica* in turfgrass areas in central New Jersey. The life cycle of *S. scarabaei* is similar to that of other *Steinernema* species (Poinar, 1990; Stock and Koppenhöfer, 2003). The free-living, non-feeding infective juveniles (IJs) seek out a host, penetrate into its body cavity, release a symbiotic bacterium, and bacteria and nematodes cooperate to overcome the host's immune response and kill it. However, host death does not occur until at least 3 days after inoculation. The bacteria propagate and protect the cadaver from colonization by other microorganisms. *S. scarabaei*-infected scarab larvae first turn a characteristic yellow-golden color but after a few days turn more yellow-brown to brown. The nematodes develop through 1–2 amphimictic generations, feeding on the bacteria and host tissues metabolized by the bacteria. Depleting food resources in the host cadaver lead to the development of a new cohort of infective juveniles that emerges from the host cadaver in search of a new host.

Our preliminary observations indicated an unusually high pathogenicity of *S. scarabaei* to several scarab species. Therefore, the major objective of this study was to determine the pathogenicity of *S. scarabaei* against third-instar *E. orientalis*, *P. japonica*, and *Cyclocephala borealis* Arrow in comparison to other scarab-pathogenic nematode species. In addition, we wanted to determine whether *S. scarabaei* would interact synergistically with imidacloprid on white grub mortality as has been already shown for other nematode species in *P. japonica*, *E. orientalis*, and the masked chafers, *Cyclocephala hirta* LeConte, *Cyclocephala pasadenae* Casey, and *C. borealis* (Koppenhöfer and Kaya, 1998; Koppenhöfer et al., 2000a, 2002).

2. Materials and methods

Field-collected third-instar larvae were used in all experiments. *E. orientalis*, *P. japonica*, and *C. borealis* were collected in turf areas at the Rutgers University Research Farm (Adelphia, NJ) and *R. majalis* were collected in turf areas at the Rutgers University Horticultural Research Farm (East Brunswick, NJ). None of the sites had been treated with insecticides during the previous year. Larvae were kept individually at 10 °C for 1–10 weeks in a mixture of organic compost and loamy sand. *S. glaseri* (NC strain), *S. glaseri* (NJ38 strain), *H. bacteriophora* (TF strain), *H. bacteriophora* (CT strain), and *Heterorhabditis* sp. (undescribed isolate from Korea) were cultured in last instars of the greater wax moth, *Galleria mellonella* (L.). *S. scarabaei* was maintained and cultured in *E. orientalis* and *P. japonica* larvae because its production in wax moth larvae is very variable with 30–70% of the cadavers not producing nematode progeny (Koppenhöfer and Fuzy, unpublished data). For one laboratory experiment, *S. scarabaei* was produced in wax moth larvae. Two other nematode isolates were maintained and cultured in the hosts they had been isolated from in the field: *H. bacteriophora* (O isolate; *E. orientalis* larvae) and *H. bacteriophora* (M isolate; *C. borealis* larvae). The emerging infective juveniles (IJs) were harvested from White traps

and stored in tap water at 10 °C (Kaya and Stock, 1997) for 6–32 days before use. Imidacloprid was obtained as a wettable powder with 75% active ingredient (AI) (Merit 75 WP; Bayer, Kansas City, MO) (recommended application rate: 330–440 g AI/ha). The soil used in the laboratory and greenhouse experiments was a sandy loam (69% sand, 14% silt, 17% clay, and 1% organic matter, pH 6.4) that had been pasteurized (3 h at 70 °C) and air-dried before use.

2.1. Laboratory experiments

Laboratory experiments were conducted at room temperature (22–26 °C) in 30-ml plastic cups (10 cm^2) filled with 25 g of moist soil with perennial ryegrass, *Lolium perenne* L., provided as food. After 3 days, individual larvae that had been held at room temperature for 24 h were released into the cups. Larvae that did not enter into the soil within 2 h were replaced. The cups were treated 1 day later. Treatments were applied in 0.5 ml water (final soil water potential −7 kPa = 12% w/w soil moisture). Controls received water only. Treatments were replicated four times with 10 cups per replicate. Experiments 1–3 each were conducted once, whereas Experiment 4 was conducted twice. Larval mortality was assessed at 7 and 14 days after treatment (DAT).

The first experiment compared the pathogenicity of *S. scarabaei*, *S. glaseri* (NC strain), *H. bacteriophora* (TF strain, M isolate, and O isolate), and *Heterorhabditis* sp. to *E. orientalis* and *C. borealis* third instars. Based on preliminary observations on nematode susceptibility of *E. orientalis* and *C. borealis*, each nematode treatment was applied at 400 IJs/cup. The second experiment compared the pathogenicity of *S. scarabaei*, *S. glaseri* (NC strain), *H. bacteriophora* (TF strain, CT strain, and O isolate), and *Heterorhabditis* sp. to *E. orientalis*, *P. japonica*, and *C. borealis* third instars. *R. majalis* third instars were exposed to *S. scarabaei*, *S. glaseri* (NC strain), and *H. bacteriophora* (TF strain and CT strain). Each nematode treatment was applied at 400 IJs/cup. The third experiment investigated the dose response of *S. scarabaei* in *E. orientalis* and *P. japonica* third instars. *S. scarabaei* dosages were 0, 6, 13, 20, 25, 50, 100, and 200 IJs/larvae for *P. japonica* and 0, 13, 20, 25, 50, 100, and 200 IJs/larva for *E. orientalis*.

A fourth laboratory experiment was conducted to determine whether the host in which the nematodes were reared for the experiments had an effect on their pathogenicity. Treatments were 20 *S. scarabaei* reared in *G. mellonella*, 20 *S. scarabaei* reared in *E. orientalis*, 400 *H. bacteriophora* TF reared in *G. mellonella*, and 400 *H. bacteriophora* TF reared in *E. orientalis*. Each nematode treatment was tested against *E. orientalis* using the same methods as in the other laboratory experiments.

2.2. Greenhouse experiments

A greenhouse experiment was conducted with *E. orientalis*, *P. japonica*, and *C. borealis* third instars. One-liter pots (10 cm × 10 cm × 10 cm; 900 cm^2 at soil surface) filled with soil to a height of 9 cm were seeded with perennial ryegrass and watered every 2–3 days until the end of the experiment. The grass was allowed to grow for 4–6 weeks and then cut using scissors before introduction of five larvae/pot. The larvae were placed on the grass 3 days before the start of an experiment. Larvae that had not entered into the soil within 24 h were replaced. The greenhouse was maintained at 28 °C/18 °C (day/night; 14/10 h L/D) and the soil temperature in the pots averaged 23.1 ± 1.6 °C. Treatments were applied in 50 ml of water. Controls received 50 ml water only.

Treatments for *E. orientalis* were (1–2) *S. glaseri* (NJ38 strain and NC strain), (3) *Heterorhabditis* sp., (4–6) *H. bacteriophora* (CT strain, TF strain, and O isolate) (treatments 1–6 each applied at 1.25×10^9 IJs/ha = 11,250/pot), (7–10) *S. scarabaei* (1.25×10^9 IJs/ha, 0.625×10^9 IJs/ha, 0.313×10^9 IJs/ha, and 0.156×10^9 IJs/ha), (11) imidacloprid (200 g AI/ha), and (12–13) the combination of imidacloprid and the two lowest *S. scarabaei* rates. Treatments for *P. japonica* were (1) *S. glaseri* (NC strain) (1.25×10^9 IJs/ha), (2–3) *H. bacteriophora* (TF strain) (1.25×10^9 IJs/ha and 0.313×10^9 IJs/ha), (4–7) *S. scarabaei* (1.25×10^9 IJs/ha, 0.625×10^9 IJs/ha, 0.313×10^9 IJs/ha, and 0.156×10^9 IJs/ha), (8) imidacloprid (200 g AI/ha), and (9) the combination of imidacloprid and the lowest *S. scarabaei* rate. Treatments for *C. borealis* were (1–2) *S. glaseri* (NJ38 strain and NC strain), (3–4) *H. bacteriophora* (O isolate and TF strain) (1.25×10^9 IJs/ha), (5–8) *S. scarabaei* (2.5×10^9 IJs/ha, 1.25×10^9 IJs/ha, 0.625×10^9 IJs/ha, and 0.313×10^9 IJs/ha), (9) imidacloprid (200 g AI/ha), and (10–11) the combination of imidacloprid and the two lowest *S. scarabaei* rates. There were 10 pots per treatment in each of two trials. After application, pots were arranged in a completely randomized design. The number of surviving larvae was determined at 14 DAT.

2.3. Field experiments

Three field experiments were conducted in areas planted with perennial ryegrass and maintained using standard management procedures at the Rutgers University Research Farm in Adelphia (Freehold, NJ). Mowing height was 3.3 cm and the thatch layer was 2-mm thick. The soils were sandy loams (65–69% sand, 18–21% silt, 13–16% clay, and 2% organic matter, pH 6.5–6.8). Preapplication sampling indicated that all sites had low resident larval populations (5–10 larvae/m^2) of *E. orientalis* (80–100%) and *P. japonica* (0–20%). None

of the detected larvae showed signs of nematode infection. Natural nematode populations were also not detected by baiting soil samples with wax moth larvae. Replicates consisted of turf microplots (0.05–0.4 m^2) surrounded by 12.5-cm high plastic barriers pushed into the soil to a depth of 11 cm. As barriers were used 25-cm diam. PVC sections or garden edging material (Emerald Edge, Easy Gardener, Waco, TX). Field-collected larvae were released into the microplots 4–7 days before application of treatments. Only larvae that entered the soil within 1 h were used. Treatments were arranged in randomized complete block designs. Applications were done as a drench in 5 mm water followed by 5–10 mm irrigation (depending on soil moisture before application), both applied using a watering can. Controls received water only.

The first experiment tested the efficacy of *S. scarabaei* against overwintered *E. orientalis* and *P. japonica* third instars. Eighty *E. orientalis* or *P. japonica* larvae were released into each microplot (0.4 m^2 surrounded by edging material). Treatments were applied on May 8, 2001 at 1000 h (soil temperature at 5-cm depth 18.5 °C; air temperature 22 °C; cloudy). There were three replicate plots per treatment. Treatments were (1–2) *S. scarabaei* (2.5×10^9 IJs/ha and 10^9 IJs/ha) and (3–4) *H. bacteriophora* (TF strain) (2.5×10^9 IJs/ha and 10^9 IJs/ha). Larval survival and infection was determined at 14 and 21 DAT by taking 5 samples per plot with a standard size golf hole cutter (0.01 m^2) to a depth of 10 cm and searching through the soil for larvae. Larvae were identified to species using the raster pattern on the lower side of their abdomen (Potter, 1998). At 14 DAT, samples including live and dead/infected larvae were carefully placed back after examination. Air temperatures averaged 15.2 °C and rainfall totaled 35 mm during the first 14 days and 15.9 °C and 58 mm during the entire experimental period, respectively. No additional overhead irrigation was supplied.

The second experiment tested the efficacy of *S. scarabaei* against third-instar *E. orientalis* in early fall and also examined whether efficacy increased over time. Twenty *E. orientalis* larvae were released into each microplot (0.1 m^2 surrounded by edging material). Treatments were applied on September 19, 2001 at 1100 h (soil temperature at 5-cm depth 24 °C; air temperature 22 °C; cloudy). There were six replicate plots per treatment. Treatments were (1–3) *S. scarabaei* (2.5×10^9 IJs/ha, 10^9 IJs/ha, and 0.4×10^9 IJs/ha), (4-5) *H. bacteriophora* (TF strain) (2.5×10^9 IJs/ha and 10^9 IJs/ha), (6) imidacloprid (330 g AI/ha), and (7) the combination of imidacloprid and the lowest *S. scarabaei* rate. Larval survival was determined at 21 and 39 DAT by searching through the soil in the microplots to a depth of 12.5 cm. Larvae were identified to species using the raster pattern on the lower side of their abdomen. Air temperatures averaged 15.3 °C and rainfall totaled 60 mm during the first 3 weeks of the experiment and 13.9 °C and 9 mm during the remainder of the experiment. No additional overhead irrigation was supplied.

The third experiment tested the efficacy of *S. scarabaei* against third-instar *C. borealis* in early fall. Twelve *C. borealis* larvae were released into each microplot (0.05 m^2 surrounded by PVC pipe sections). Treatments were applied on September 25, 2001 at 1500 h (soil temperature at 5-cm depth 23 °C; air temperature 22 °C; sunny). There were eight replicate plots per treatment. Treatments were (1–2) *S. scarabaei* (2.5×10^9 IJs/ha and 10^9 IJs/ha) and (3–4) *H. bacteriophora* (TF strain) (2.5×10^9 IJs/ha and 10^9 IJs/ha). Larval survival was determined at 21 DAT by searching through the soil in the microplots to a depth of 12.5 cm. Larvae were identified to species using the raster pattern on the lower side of their abdomen. During the experiment, air temperatures averaged 15.3 °C and rainfall totaled 38 mm. No additional overhead irrigation was supplied.

2.4. Statistics

Each experiment except for the fourth laboratory experiment and the greenhouse experiment was conducted once. The number of surviving or infected larvae was square root transformed and analyzed using ANOVA and means were separated with Tukey's test (SAS Institute, 1996). The dosage mortality data for *P. japonica* and *E. orientalis* were analyzed using probit analysis. Synergistic, additive, or antagonistic interactions between agents in the combination treatments were determined using a χ^2 test (Finney, 1964; Koppenhöfer and Kaya, 1998; McVay et al., 1977). Grub mortality was calculated by subtracting the number of surviving grubs from the number of grubs released for each replicate and correcting for control mortality (Abbott, 1925). In the field experiment, grub mortality was calculated by subtracting the number of detected grubs in a treatment plot from the mean number of grubs recovered in the control. The expected additive proportional mortality M_E for the nematode–imidacloprid combinations was calculated by $M_E = M_N + M_I(1 - M_N)$, where M_N and M_I are the observed proportional mortalities caused by nematodes and imidacloprid alone, respectively. Results from a χ^2 test, $\chi^2 = (M_{NI} - M_E)^2/M_E$, where M_{NI} is the observed mortality for the nematode–imidacloprid combinations, were compared to the χ^2 table value for 1 df. If the calculated χ^2 value exceeded the table value, a non-additive effect between the two agents was suspected (Finney, 1964). If the difference $M_{NI} - M_E$ had a positive (negative) value, a significant interaction was then considered synergistic (antagonistic). Differences among means in all experiments were considered significant at $P < 0.05$. Means ± SE are presented.

3. Results

3.1. Laboratory experiments

In the first experiment, there was no significant effect of scarab species at 7 DAT but a significant effect at 14 DAT ($F = 4.9$; df $= 1, 46$; $P = 0.03$). There was a significant effect of nematode species and a significant interaction between scarab species and nematode species at 7 and 14 DAT ($F > 16.2$; df $= 5, 42$; $P < 0.001$). Accordingly, comparisons were made by scarab species and by nematode species. Third-instar mortality differed significantly among treatments at 7 and 14 DAT in *E. orientalis* ($F \geqslant 20.9$; df $= 5, 18$; $P < 0.001$) (Fig. 1A) and *C. borealis* ($F \geqslant 8.4$; df $= 5, 18$; $P < 0.001$) (Fig. 1B). There was no control mortality. In *E. orientalis*, only *S. scarabaei* was highly pathogenic with 98% mortality at 14 DAT. All other isolates were significantly less pathogenic with 25–62% mortality at 14 DAT. In *C. borealis*, no nematode isolate was highly pathogenic. *S. scarabaei*, *H. bacteriophora* (TF strain), *H. bacteriophora* (O isolate), and *Heterorhabditis* sp. were moderately pathogenic with 53–62% mortality at 14 DAT. *H. bacteriophora* (M isolate), even though isolated from and maintained in *C. borealis*, was intermediate in pathogenicity with 35% mortality at 14 DAT. *S. glaseri* NC was the least pathogenic isolate with 18% mortality at 14 DAT.

Relative nematode susceptibility of *E. orientalis* and *C. borealis* in the first experiment varied with nematode isolate. Thus, *E. orientalis* was more susceptible than *C. borealis* to *S. scarabaei* (7 and 14 DAT: $F \geqslant 51.2$; df $= 1, 6$; $P < 0.001$) and *S. glaseri* (NC strain) ($F \geqslant 25.3$; df $= 1, 6$; $P < 0.001$). There was no difference between *E. orientalis* and *C. borealis* in susceptibility to *Heterorhabditis* sp. (7 and 14 DAT: $P \geqslant 0.63$), *H. bacteriophora* (M isolate) (7 and 14 DAT: $P \geqslant 0.09$), and *H. bacteriophora* (TF strain) (7 and 14 DAT: $P \geqslant 0.06$). Finally, *E. orientalis* was less susceptible than *C. borealis* to *H. bacteriophora* (O isolate) (7 and 14 DAT: $F \geqslant 15.2$; df $= 1, 6$; $P < 0.01$).

In the second experiment, there was a significant effect of scarab species ($F \geqslant 87.5$; df $= 3, 100$; $P < 0.001$) and nematode species ($F \geqslant 111.2$; df $= 6, 97$; $P < 0.001$) and a significant interaction between scarab species and nematode species ($F \geqslant 15.0$; df $= 16, 81$; $P < 0.001$) at 7 and 14 DAT. Accordingly, comparisons were made by scarab species and by nematode species. Third-instar mortality differed significantly among treatments at 7 and 14 DAT in *P. japonica* ($F \geqslant 36.9$; df $= 6, 21$; $P < 0.001$), *E. orientalis* ($F \geqslant 40.8$; df $= 6, 21$; $P < 0.001$), *C. borealis* ($F \geqslant 14.1$; df $= 6, 21$; $P < 0.001$),

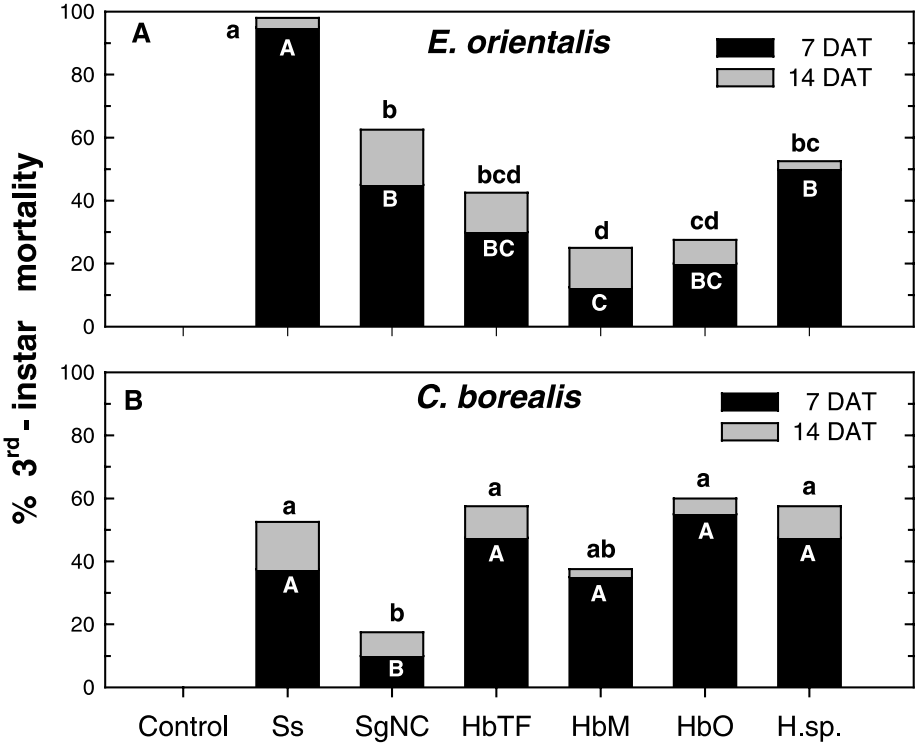

Fig. 1. Effect of treatment with the entomopathogenic nematodes *S. scarabaei* (Ss), *S. glaseri* (NC strain) (SgNC), *H. bacteriophora* (TF strain) (HbTF), *H. bacteriophora* (M isolate) (HbM), *H. bacteriophora* (O isolate) (HbO), and *Heterorhabditis* sp. (H.sp.) (each at 400 IJ/cup) on mortality (mean ± SE) of third-instar *E. orientalis* (A) and *C. borealis* (B) in 30-ml cups with soil and grass. Capital letters in black section of bar indicate significant differences in mortality at 7 DAT, lower case letters above bars indicate significant differences in mortality at 14 DAT ($P < 0.05$).

and *R. majalis* ($F \geqslant 75.9$; df $= 4, 15$; $P < 0.001$). Control mortality was low in all four scarab species (3–8% at 14 DAT). *P. japonica* was highly susceptible to all nematode isolates with 80–100% mortality at 14 DAT. At 7 DAT, *S. scarabaei* and *H. bacteriophora* (O isolate) caused the highest mortality (96–100%), whereas *S. glaseri* (NC strain) caused the lowest mortality (60%) (Fig. 2A). *E. orientalis* mortality followed a very similar pattern as in the first experiment with *S. scarabaei* causing 98% mortality at 14 DAT, and all other isolates causing low to moderate mortality (18–45% at 14 DAT) (Fig. 2B). Similarly, mortality of *R. majalis* larvae was 100% at 14 DAT for *S. scarabaei* but 25–55% for all other nematode isolates (Fig. 2D). *C. borealis* mortality followed a

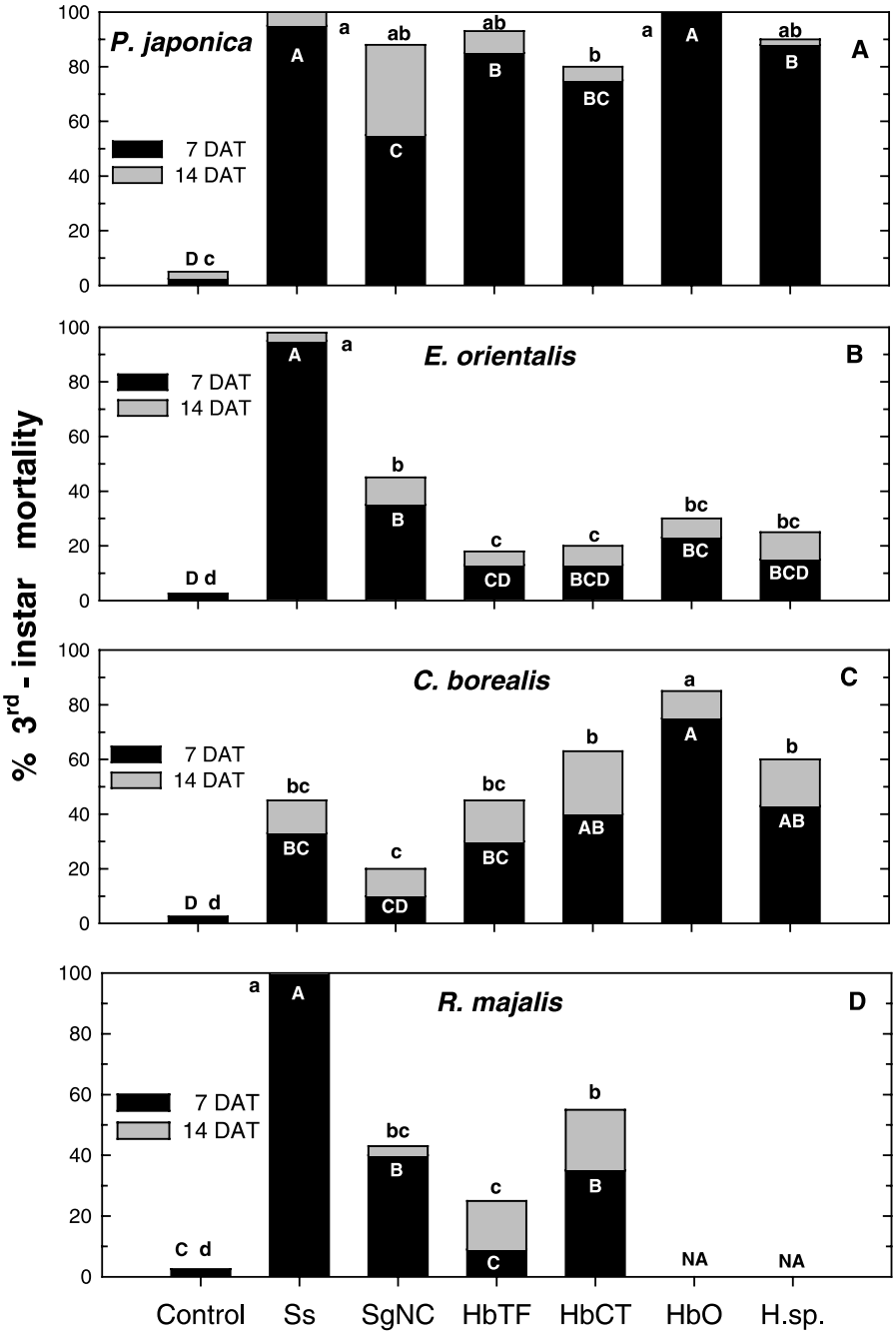

Fig. 2. Effect of treatment with the entomopathogenic nematodes *S. scarabaei* (Ss), *S. glaseri* (NC strain) (SgNC), *H. bacteriophora* (TF strain) (HbTF), *H. bacteriophora* (CT strain) (HbCT), *H. bacteriophora* (O isolate) (HbO), and *Heterorhabditis* sp. (H.sp.) (each at 400 IJ/cup) on mortality (mean ± SE) of third-instar *P. japonica* (A), *E. orientalis* (B), *C. borealis* (C), and *R. majalis* (D) in 30-ml cups with soil and grass. Capital letters in black section of bar indicate significant differences in mortality at 7 DAT, lower case letters above bars indicate significant differences in mortality at 14 DAT ($P < 0.05$). NA, treatment was not included.

similar pattern as in the first experiment with moderate mortality caused by *S. scarabaei*, *Heterorhabditis* sp., and the TF and CT strains of *H. bacteriophora* (45–60% at 14 DAT), and low mortality for *S. glaseri* (NC strain) (20% at 14 DAT). The only treatment causing high mortality was *H. bacteriophora* (O isolate) (85% at 14 DAT) (Fig. 2C).

Relative nematode susceptibility among scarab species in the second experiment varied with nematode isolate. *Heterorhabditis* sp. and *H. bacteriophora* (O isolate) caused the highest mortality in *P. japonica*, significantly less mortality in *C. borealis*, and the lowest mortality in *E. orientalis* (7 and 14 DAT: $F \geqslant 30.8$; df $= 2, 9$; $P < 0.001$) (not tested against *R. majalis*). *H. bacteriophora* (CT strain) caused the highest mortality in *P. japonica*, significantly less mortality in *C. borealis* and *R. majalis,*and the lowest mortality in *E. orientalis* (7 and 14 DAT: $F \geqslant 18.6$; df $= 3, 12$; $P < 0.001$). *H. bacteriophora* (TF strain) caused the highest mortality in *P. japonica*, significantly less mortality in *C. borealis*, and the lowest mortality in *E. orientalis*, with *R. majalis* falling between *C. borealis* and *E. orientalis* (7 and 14 DAT: $F \geqslant 24.4$; df $= 3, 12$; $P < 0.001$). *S. glaseri* (NC strain) caused significantly higher mortality in *P. japonica*, *R. majalis*, and *E. orientalis* than in *C. borealis* at 7 DAT ($F = 10.1$; df $= 3, 12$; $P < 0.001$) and significantly higher mortality in *P. japonica* than in *R. majalis*, *E. orientalis*, and *C. borealis* at 14 DAT ($F = 17.8$; df $= 3, 12$; $P < 0.001$). Finally, *S. scarabaei* caused significantly higher mortality in *P. japonica*, *R. majalis*, and *E. orientalis* than in *C. borealis* at 7 and 14 DAT ($F \geqslant 75.3$; df $= 3, 12$; $P < 0.001$).

In the third experiment, *E. orientalis* mortality by *S. scarabaei* increased significantly in a dose response at 7 and 14 DAT ($F \geqslant 30.4$; df $= 5, 18$; $P < 0.001$). At a dose of 50–200 IJs/larva, mortality was 95–100% at 14 DAT (Fig. 3A). Using the dosage range of 0–50 IJs, the LC50 (95% fiducial limits) at 14 DAT was 17.9 (15.6–20.2) IJs/larva. The LC90 (95% fiducial limits) at 14 DAT was 35.2 (29.6–48.0) IJs/larva. *P. japonica* mortality also increased significantly with dosage at 7 and 14 DAT ($F \geqslant 32.2$; df $= 6, 21$; $P < 0.001$). At a dose of 20–200 IJs/larva, mortality was 90–100% at 14 DAT (Fig. 3B). Using the dosage range of 0–50 IJs, the LC50 (95% fiducial limits) at 14 DAT was 10.9 (5.8–15.5) IJs/larva. The LC90 (95% fiducial limits) at 14 DAT was 22.3 (15.6–67.8) IJs/larva. Based on the lack of overlap of LC50 fiducial limits at 7 DAT (data not shown) and 14 DAT, *S. scarabaei* was more pathogenic to *P. japonica* than *E. orientalis*.

In the fourth laboratory experiment, there was no effect of trial and the data were combined for analysis.

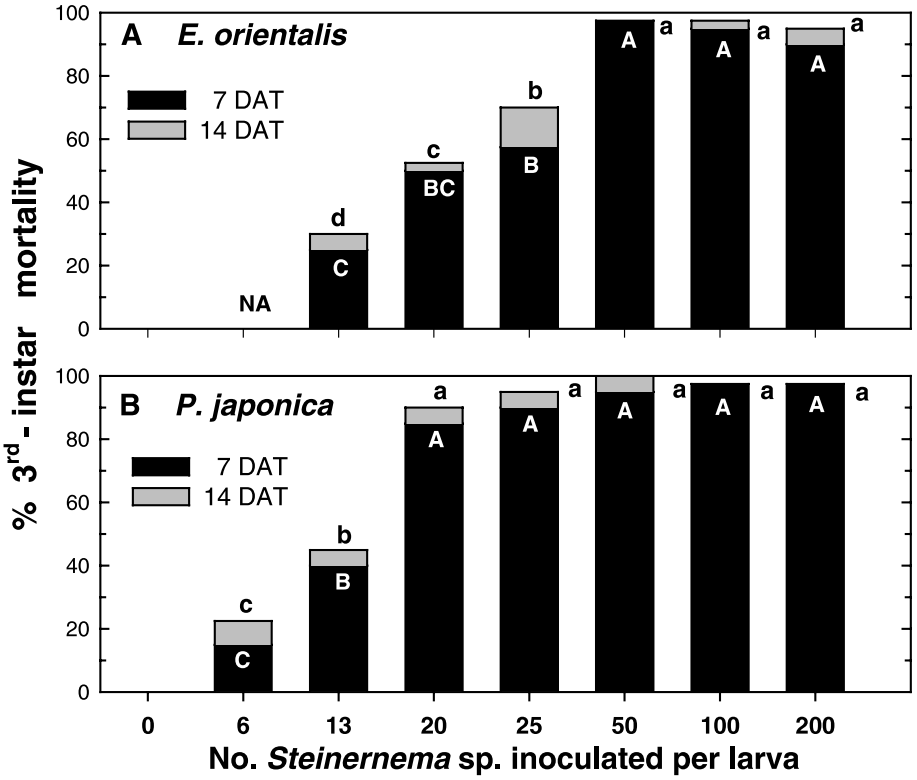

Fig. 3. Effect of treatment with different doses of the entomopathogenic nematode *S. scarabaei* on mortality (mean ± SE) of third-instar *P. japonica* (A) and *E. orientalis* (B) in 30-ml cups with soil and grass. Capital letters in black section of bar indicate significant differences in mortality at 7 DAT, lower case letters above bars indicate significant differences in mortality at 14 DAT ($P < 0.05$). NA, treatment was not included.

Table 1
Effect of rearing host on nematode pathogenicity to third-instar *E. orientalis* in the laboratory

	S. scarabaei (20 IJs[a] /larva)			*H. bacteriophora* (400 IJs/larva)		
Host	*E. orientalis*	*G. mellonella*	*P*	*E. orientalis*	*G. mellonella*	*P*
7 DAT[b]	34 ± 7	52 ± 7	0.03	30 ± 8	36 ± 8	0.43
14 DAT	53 ± 8	66 ± 9	0.10	44 ± 8	45 ± 10	0.89

There was no control mortality. Mean mortality was compared using *t* test. Data shown are means ± SE.
[a] Infective juvenile nematodes.
[b] Days after treatment.

Host species in which the nematodes were reared had no effect on the pathogenicity of *H. bacteriophora* TF strain to third-instar *E. orientalis* (Table 1). In *S. scarabaei*, pathogenicity to third-instar *E. orientalis* tended to be higher when it was reared in *G. mellonella* than when it was reared in *E. orientalis* but the effect was significant only at 7 DAT and not at 14 DAT (Table 1).

3.2. Greenhouse experiment

There was no effect of trial but a significant effect of scarab species ($F = 150.1$; df $= 2, 717$; $P < 0.001$) and nematode species ($F = 67.8$; df $= 16, 703$; $P < 0.001$) and a significant interaction of nematode species and scarab species ($F = 19.3$; df $= 17, 702$; $P < 0.001$). Accordingly data of the two trials were combined and analyzed by scarab species and by nematode species. In *E. orientalis*, there were significant differences in larval mortality among treatments ($F = 38.48$; df $= 27, 252$; $P < 0.001$) (Fig. 4A). All nematode isolates but *S. scarabaei* caused low to moderate mortality (28–47%). *S. scarabaei* showed a dose response with 65–96% mortality at 0.156–1.25×10^9 IJs/ha. But even the lowest rate of *S. scarabaei* caused significantly higher mortality than any of the other nematode isolates except for *H. bacteriophora* (O isolate) (47%). Imidacloprid alone caused low mortality (14%) but interacted synergistically with *S. scarabaei* in both combination treatments (90–94% mortality) ($\chi^2 \geqslant 4.0$; df $= 1$; $P \leqslant 0.03$).

In *P. japonica*, there were significant differences in larval mortality among treatments ($F = 38.48$; df $= 27, 252$; $P < 0.001$) (Fig. 4B). All nematode isolates caused high (82–95%) mortality. There was no significant decrease in mortality from 1.25×10^9 to 0.325×10^9 IJs/ha for both *S. scarabaei* and *H. bacteriophora* (TF strain). At the lowest *S. scarabaei* rate (0.156×10^9 IJs/ha) mortality decreased significantly but was still at 70%. Imidacloprid alone caused 30% mortality but there was no interaction with *S. scarabaei* in the combination treatment.

In *C. borealis*, there were significant differences in larval mortality among treatments ($F = 38.48$; df $= 27, 252$; $P < 0.001$) (Fig. 4C). At a rate of 1.25×10^9 IJs/ha, *S. scarabaei* caused significantly higher mortality (69%) than *H. bacteriophora* (TF strain) (49%) but not significantly higher mortality than *H. bacteriophora* (O isolate) (60%). Both *S. glaseri* strains (NJ38 and NC) were ineffective (14–15% mortality). *S. scarabaei* showed a clear dose response with 44–75% mortality at 0.313×10^9–2.5×10^9 IJs/ha. Imidacloprid alone caused 13% mortality but there was no interaction with *S. scarabaei* in the combination treatments.

Relative nematode susceptibility among scarab species varied with nematode isolate. *H. bacteriophora* (O isolate) caused significantly higher mortality to *C. borealis* than *E. orientalis* ($F = 4.3$; df $= 1, 38$; $P = 0.04$) (not tested against *P. japonica*). *H. bacteriophora* (TF strain) cause the highest mortality to *P. japonica*, significantly less in *C. borealis*, and the lowest mortality in *E. orientalis* ($F = 103.8$; df $= 2, 57$; $P < 0.001$). *S. glaseri* (NJ38 strain) caused significantly higher mortality in *E. orientalis* than in *C. borealis* ($F = 17.7$; df $= 1, 38$; $P < 0.001$) (*P. japonica* not tested), whereas mortality caused by *S. glaseri* (NC strain) did not differ significantly between *E. orientalis* and *C. borealis* but was higher than in these two species in *P. japonica* ($F = 69.5$; df $= 2, 57$; $P < 0.001$). Susceptibility among scarab species to *S. scarabaei* varied with the different application rates. At 1.25×10^9 and 0.625×10^9 IJs/ha, *S. scarabaei* caused significantly higher mortality in *P. japonica* and *E. orientalis* than in *C. borealis* ($F \geqslant 19.5$; df $= 2, 57$; $P < 0.001$). At 0.313×10^9 IJs/ha, *S. scarabaei* caused the highest mortality in *P. japonica*, significantly less mortality in *E. orientalis*, and the lowest mortality in *C. borealis* ($F = 69.5$; df $= 2, 57$; $P < 0.001$). At 0.156×10^9 IJs/ha, *S. scarabaei*-caused mortality did not differ significantly between *P. japonica* and *E. orientalis* (*C. borealis* not tested).

3.3. Field experiments

In the first experiment with overwintered *P. japonica* and *E. orientalis*, an average of 10.2 ± 0.5 larvae (range 8–12) were recovered in the 5 cores taken from the control of both species at 14 DAT and 21 DAT. One nematode-infected larva was found in the controls and had the typical red-brown color of an *H. bacteriophora*-infected larva. Because there were significant effects of evaluation date (more mortality at 21 DAT) and scarab species (more mortality in *P. japonica*) ($F \geqslant 8.5$; df $=$

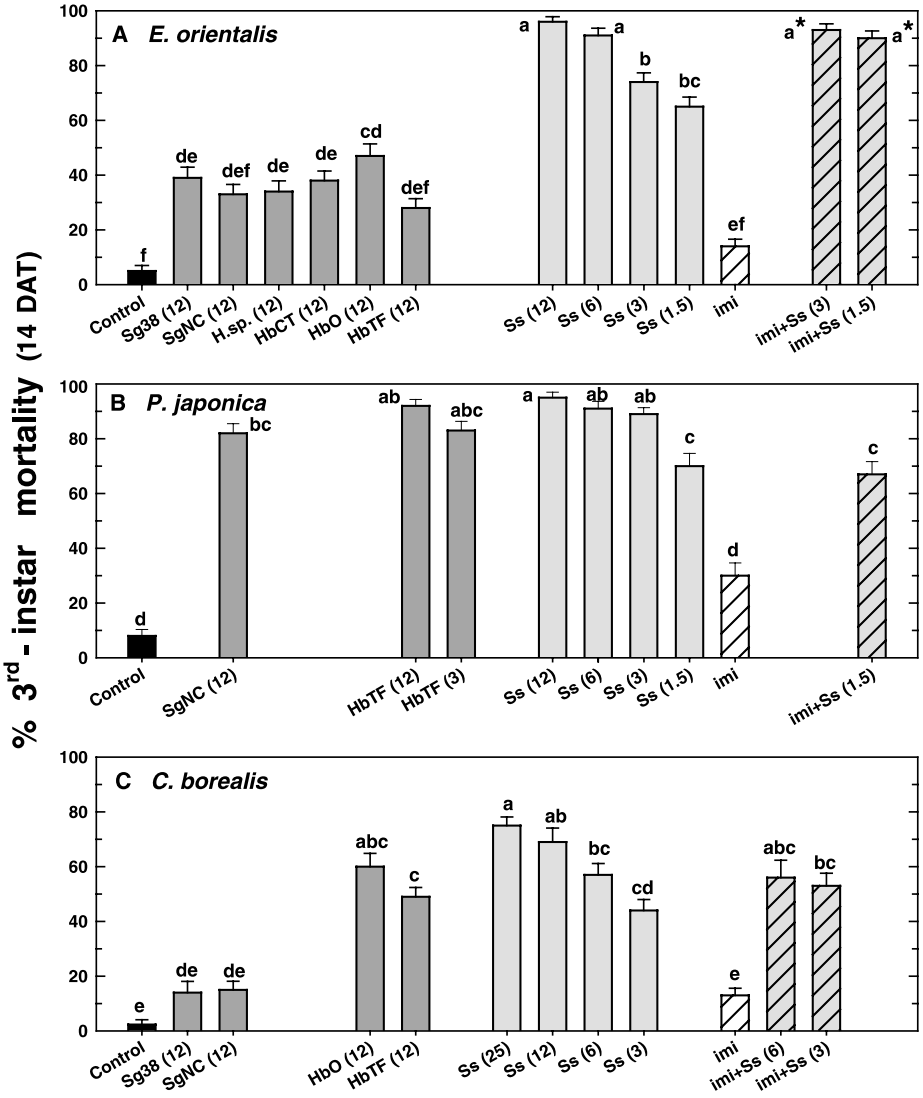

Fig. 4. Effect of treatment with the entomopathogenic nematodes *S. scarabaei* (Ss), *S. glaseri* (NC strain) (SgNC), *S. glaseri* (NJ38 strain) (Sg38), *Heterorhabditis* sp. (H.sp.), *H. bacteriophora* (CT strain) (HbCT), *H. bacteriophora* (O isolate) (HbO), and *H. bacteriophora* (TF strain) (HbTF), the neonicotinoid imidacloprid (imi), and the combination of imidacloprid with *S. scarabaei* on mortality (mean ± SE) of third-instar *E. orientalis* (A), *P. japonica* (B), and *C. borealis* (C) in 1-L pots with grass. Figures in brackets indicate nematode dose: 1.5, 3, 6, 12, and 25 correspond to 0.156, 0.313, 0.625, 1.25, and 2.5×10^9 IJs/ha. To facilitate comparisons among species, same treatments are placed at the same position on the *x*-axis of each scarab species. Means with same letter are not significantly different ($P < 0.05$). An '*' indicates significant synergistic interactions between nematode and imidacloprid.

$1, 58$; $P < 0.01$) and significant interactions between treatment and scarab species and treatment and evaluation day ($F \geqslant 2.6$; df $= 4, 55$; $P < 0.05$), the data were analyzed by day and scarab species or nematode treatment.

Significant differences among treatments in the first experiment were found for mortality at 14 DAT and 21 DAT in both scarab species (Fig. 5A and B) and for percentage larvae infected in *P. japonica* ($F \geqslant 5.7$; df $= 4, 10$; $P < 0.001$) (Fig. 5C) but not in *E. orientalis* (Fig. 5D). In *P. japonica*, both *S. scarabaei* rates provided 100% control at 14 and 21 DAT and all cadavers recovered were infected with *S. scarabaei*. *H. bacteriophora* (TF strain) provided 74% (93%) control at 14 (21) DAT at the higher rate (2.5×10^9 IJ/ha) (not significantly different from *S. scarabaei*), but only 33% (40%) control at 14 (21) DAT at the lower rate (10^9 IJ/ha) (significantly less than *S. scarabaei*). Data for percentage infection at 14 DAT followed very closely the mortality data (Fig. 5C). In *E. orientalis*, only *S. scarabaei* provided excellent control at 14 DAT (80–94%) and 21 DAT (93–97%) (Fig. 5B). *H. bacteriophora* (TF strain) activity was obviously delayed compared to *S. scarabaei* with no control at 14 DAT (0–7%) but moderate control at 21 DAT (40–53%). Percentage infection data at 14 DAT followed very closely the mortality data (Fig. 5D).

Relative nematode susceptibility of *E. orientalis* and *P. japonica* in the first field experiment varied with

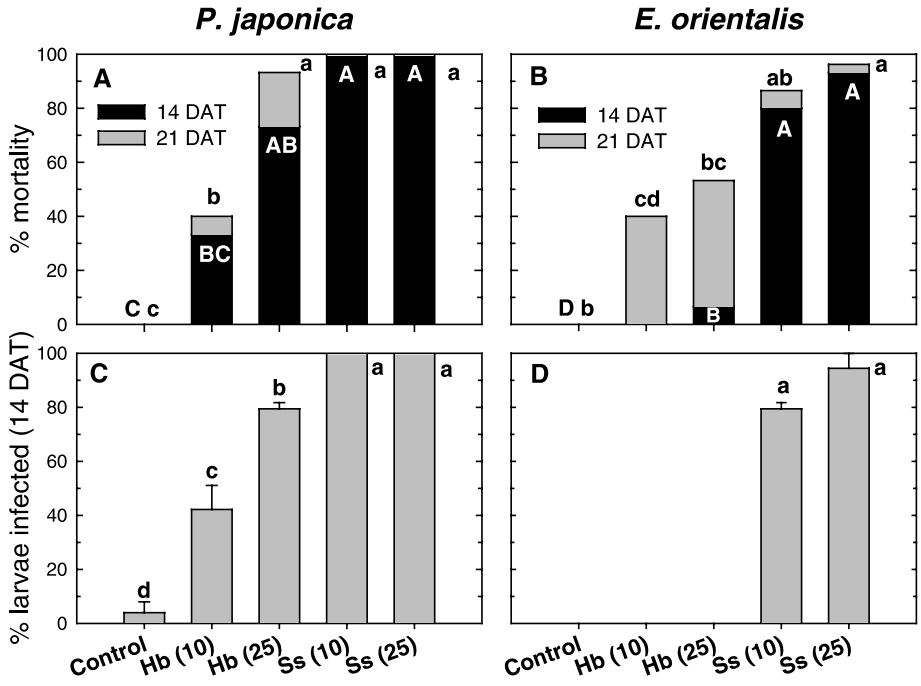

Fig. 5. Effect of treatment with the entomopathogenic nematode *S. scarabaei* (Ss) and *H. bacteriophora* (TF strain) (Hb) [each applied at 10^9 IJs/ha (10) and 2.5×10^9 IJs/ha (25)] on mortality (A, B) and percentage nematode-infected larvae (C, D) (mean ± SE) of third-instar *P. japonica* (A, C) and *E. orientalis* (B, D) in 0.4 m^2 microplots surrounded by garden edging material in a turfgrass field. Capital letters in black section of bar indicate significant differences in mortality at 14 DAT, lower case letters above bars indicate significant differences in mortality at 21 DAT or percent infection at 14 DAT ($P < 0.05$).

nematode species. *S. scarabaei* caused higher mortality in *P. japonica* than in *E. orientalis* only at 10^9 IJ/ha and at 14 DAT ($F = 100.2$; df $= 1, 4$; $P < 0.001$). At the higher rate (2.5×10^9 IJ/ha) at 14 DAT and at both rates at 21 DAT, the high mortality in all treatments ($\geqslant 93\%$) did not allow for a differentiation between scarab species. *H. bacteriophora* caused higher mortality in *P. japonica* than in *E. orientalis* at the higher rate at 14 DAT ($F = 50.0$; df $= 1, 4$; $P < 0.001$) and 21 DAT ($F = 7.8$; df $= 1, 4$; $P < 0.05$) and at the lower rate at 14 DAT ($F = 7.8$; df $= 1, 4$; $P < 0.05$) but not at 21 DAT.

In the second experiment testing *S. scarabaei* efficacy against *E. orientalis*, an average of 31.0 ± 3.0 and 25.5 ± 3.9 *E. orientalis* larvae were recovered in the controls at 21 DAT and 39 DAT, respectively. Because only 20 larvae had been released before treatment, it was obvious that we had missed several areas with high larval populations during the pretreatment sampling. On both evaluation dates, one nematode-infected larva was found in the controls and both showed the typical red-brown color of a *H. bacteriophora*-infected larva. Differences among treatments were significant at 21 DAT and 39 DAT ($F \geqslant 13.0$; df $= 12, 35$; $P < 0.001$) (Fig. 6). At 21 DAT, *S. scarabaei* showed a clear dose–response with 43–85% control at the three application rates (0.4–2.5×10^9 IJ/ha). *S. scarabaei* provided significantly better control than *H. bacteriophora* (TF strain) (85% vs. 45% at 2.5×10^9 IJs/ha; 60% vs. 11% at 10^9 IJs/ha). Imidacloprid alone only provided 33% control and there was no significant interaction with the lowest *S. scarabaei* rate in the combination treatment. At 39 DAT, control rates followed a similar pattern as after 21 DAT, however, differences were not as clear because, compared to the 21 DAT observations, the lower rates

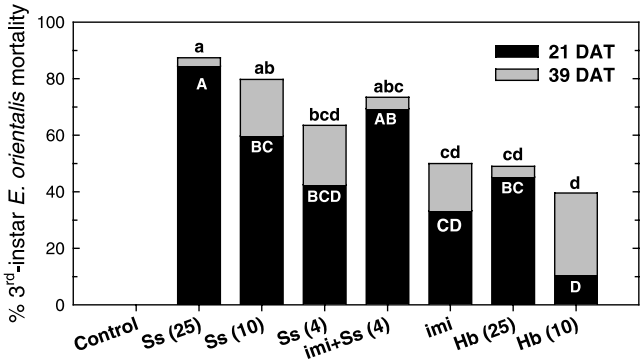

Fig. 6. Effect of treatment with the entomopathogenic nematode *S. scarabaei* (Ss) and *H. bacteriophora* (TF strain) (Hb), the neonicotinoid imidacloprid (330 g AI/ha) (imi), and the combination of imidacloprid and the lowest *S. scarabaei* rate (imi + Ss) on mortality (mean ± SE) of third-instar *E. orientalis* in 0.1 m^2 microplots surrounded by garden edging material in a turfgrass field. Figures in brackets indicate nematode dose: 4, 10, and 25 correspond to 0.4, 1.0, and 2.5×10^9 IJs/ha. Capital letters in black section of bar indicate significant differences in mortality at 21 DAT, lower case letters above bars indicate significant differences in mortality or percent infection at 39 DAT ($P < 0.05$).

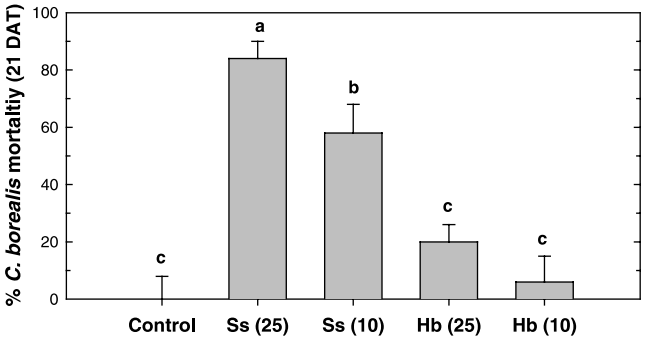

Fig. 7. Effect of treatment with the entomopathogenic nematode *S. scarabaei* (Ss) and *H. bacteriophora* (TF strain) (Hb) [each applied at 10^9 IJs/ha (10) and 2.5×10^9 IJs/ha (25)] on mortality (mean ± SE) of third-instar *C. borealis* in 0.05 m^2 microplots surrounded by PVC pipe sections in a turfgrass field. Means with same letter are not significantly different ($P < 0.05$).

of both nematode species and imidacloprid had an increase of 17–29% in control whereas the highest rate of both nematode species and the combination treatment only had an increase of 3–4% in control. It has to be noted that only 9-mm rainfall were recorded during the second half of the experiment which may have restricted any additional control by the nematodes.

In the third experiment testing *S. scarabaei* efficacy against *C. borealis*, an average of 8.0 ± 0.6 (range 6–10) *C. borealis* larvae and 0.9 ± 0.3 (range 0–2) *E. orientalis* larvae were recovered from the controls. *E. orientalis* numbers were too low for analysis and meaningful interpretation. Number of *C. borealis* larvae recovered varied significantly among treatments ($F = 12.6$; $df = 9, 20$; $P < 0.001$) (Fig. 7). The highest *S. scarabaei* rate provided significantly higher control (84%) than the lower *S. scarabaei* rate (58%), which in turn provided significantly higher control than the higher (20%) and lower (6%) *H. bacteriophora* (TF strain) rate.

4. Discussion

This study shows that *S. scarabaei*, a new entomopathogenic nematode species (Stock and Koppenhöfer, 2003) recently isolated from epizootics in larval populations of *E. orientalis* and *P. japonica*, has exceptional potential as a biological control agent of white grubs. Under field conditions, *S. scarabaei* outperformed *H. bacteriophora* in *E. orientalis*, *C. borealis*, and *P. japonica* not only with respect to overall control rate but also with respect to speed of kill. It is interesting to note that the efficacy of *S. scarabaei* relative to the other scarab-pathogenic nematode species tested improved from the laboratory bioassays over the greenhouse pot experiments to the field microplots. *S. scarabaei* appears to be not only very well adapted (high pathogenicity, infectivity, and reproductive potential; cruiser type foraging behavior) but rather specialized to scarab larvae as hosts (laboratory host range) (Koppenhöfer and Fuzy, 2003). We hypothesize the superiority of *S. scarabaei* as a white grub control agent through this high degree of adaptation may be more clearly manifested under field conditions than under laboratory conditions where nematode–host contact is assured. Whether this specificity can also translate into better long-term control of white grubs in the form of inoculative or periodic augmentative releases, needs to be clarified. The increase in *E. orientalis* control between 21 DAT and 39 DAT in one of our field experiments was somewhat limited, but may have been restricted by the dry conditions during the second half of the experimental period (Georgis and Gaugler, 1991; Shetlar et al., 1988). In a more recent field experiment, *S. scarabaei* provided 100% control of *E. orientalis* at 31 DAT even at a rate of 0.4×10^9/ha (Koppenhöfer and Fuzy, unpublished data). Based on the here presented data such high efficacy suggests additional grub mortality caused by the *S. scarabaei* progeny emerged from grubs infected by the originally applied nematodes.

Our observations show that nematode efficacy against white grubs varies considerably with nematode species/strain and white grub species. Among the white grub species examined, *P. japonica* was by far the most nematode susceptible species with high susceptibility to all nematode isolates tested. Only under field conditions and at low nematode application rate was the superiority of *S. scarabaei* over *H. bacteriophora* apparent. In a direct and repeated comparison in a different study (Koppenhöfer and Fuzy, unpublished data), third-instar *P. japonica* mortality was 80–100% when exposed to 20 *S. scarabaei* but only 50–70% when exposed to 100 *H. bacteriophora* (TF strain). *C. borealis* and *E. orientalis* were considerably less nematode-susceptible than *P. japonica* but differed in their response to different nematode species. Under laboratory and greenhouse conditions, *C. borealis* showed moderate susceptibility to all nematodes tested including *S. scarabaei* and low susceptibility to *S. glaseri*. But in the field experiment, *S. scarabaei* showed excellent efficacy with 4–9 times higher control than *H. bacteriophora* (TF strain). In contrast, *E. orientalis* was highly susceptible to *S. scarabaei* but showed moderate to low susceptibility to all other nematodes. *R. majalis* and *M. castanea* follow a similar pattern in nematode-susceptibility as *E. orientalis*, except that *R. majalis* may be somewhat more nematode-susceptible (this study; Cappaert and Koppenhöfer, unpublished data) and *M. castanea* is less nematode-susceptible than *E. orientalis* (Koppenhöfer and Fuzy, in press).

In considering the superiority of *S. scarabaei* as a white grub control agent, it has to be noted that only a limited number of nematode strains/spp. were compared to *S. scarabaei* in our study, especially under field

conditions. In a laboratory study Grewal et al. (2002) found considerable variation among different strains of *H. bacteriophora* and some of those strain may have performed better than the strains tested in our study against *P. japonica* or *C. borealis*. The same may be true for *Heterorhabditis zealandica* Poinar but probably not for *Heterorhabditis megidis* Poinar, Jackson and Klein and certainly not for *H. marelatus* Liu and Berry and *H. indica* Poinar, Karunakar and David (Grewal et al., 2002). *H. indica* also performed very poorly in a field trial against *P. japonica* (Koppenhöfer et al., 2000a). The superiority of *S. scarabaei* against *E. orientalis*, however, is much clearer than in *P. japonica* and *C. borealis* and it is unlikely that other *H. bacteriophora* strains would come even close in efficacy to that of *S. scarabaei*. *H. marelatus* and *H. megidis* were not compared to *S. scarabaei* in the present study, but in previous greenhouse experiments (Koppenhöfer et al., 2002) these nematodes did not perform better than *H. bacteriophora* (TF strain) against *E. orientalis*.

It has to be noted that Grewal et al. (2002) did not observe the same differences in nematode-susceptibility as we report here. While *R. majalis* showed a low nematode-susceptibility as observed in the present study, there was no clear difference between *P. japonica*, *C. borealis*, and *E. orientalis*. Overall, in the Grewal et al. (2002) study, *P. japonica* appeared to be considerably less nematode-susceptible than in the present study whereas *E. orientalis* appeared to be more nematode-susceptible. However, comparisons between the two studies have to be done with caution because different nematode strains were used. Such differences in nematode-susceptibility as apparent between the two studies may have several reasons including different age of grubs at time of collection, different storage conditions and length, or maybe even differences between grubs collected from different regions.

At this point we can only speculate about the factors responsible for the interactions among nematode and scarab species observed in the present study. As a result of their coevolution with entomopathogenic nematodes and other pathogens in the soil, white grubs possess a variety of defense mechanisms including infrequent CO_2 output, sieve plates over their spiracles, frequent defecation, defensive and evasive behaviors, a dense peritrophic membrane, and a strong immune response. However, these defense mechanisms have only been described for some scarab species, and it is likely that their occurrence and degree of expression varies considerably among scarab species. For example, *P. japonica* larvae show much stronger defensive and evasive behaviors against entomopathogenic nematodes than *Cyclocephala* spp. larvae (Gaugler et al., 1994; Koppenhöfer et al., 2000b), yet *Cyclocephala* spp. larvae are less nematode-susceptible suggesting differences in other defense mechanisms.

Culture method can have an effect on nematode pathogenicity. In our study, *S. scarabaei* and two *H. bacteriophora* strains were reared in *E. orientalis* for most experiments whereas all other nematodes were reared in *G. mellonella*. However, *S. scarabaei* did not gain an advantage by being reared in *E. orientalis*. Rather *S. scarabaei* tended to be more pathogenic to *E. orientalis* when reared in *G. mellonella* than when reared in *E. orientalis*. In the same experiment, *H. bacteriophora* (TF strain) pathogenicity to *E. orientalis* was not affected by rearing host. Gaugler and Georgis (1991) observed that *H. bacteriophora* gave better levels of control of *P. japonica* and *C. borealis* when reared in *G. mellonella* and on solid media than when reared in liquid culture, whereas *Steinernema carpocapsae* (Weiser) efficacy against *P. japonica* and *Otiorhynchus sulcatus* (F.) was not affected by culture method (Gaugler and Georgis, 1991). *Steinernema scapterisci* Nguyen and Smart were more pathogenic to *G. mellonella* when reared in house crickets, *Acheta domesticus* (L.), than when reared in liquid culture, whereas *S. carpocapsae* pathogenicity to *G. mellonella* was not affected by culture method (Grewal et al., 1999). *S. glaseri* infectivity to *G. mellonella* was not affected by rearing host (*G. mellonella* vs. *P. japonica*) (Stuart and Gaugler, 1996).

Our observations on the interaction between *S. scarabaei* and imidacloprid were variable. We observed a consistent but relatively weak synergistic interaction in *E. orientalis* in two greenhouse trials but no synergism in a field experiment, and there was no synergism in the greenhouse experiment with *P. japonica* and *C. borealis*. It is not surprising that nematode–imidacloprid interaction varies between nematode species. Thus, imidacloprid has already shown a generally stronger interaction with *S. glaseri* than with *H. bacteriophora* and no interaction with *Steinernema kushidai* (Koppenhöfer et al., 2000a, 2002). It is not clear why *S. scarabaei* interacted with imidacloprid in *E. orientalis* but not in the more nematode-susceptible *P. japonica* or the less nematode-susceptible *C. borealis*. In previous studies, imidacloprid interacted synergistically with both *S. glaseri* and *H. bacteriophora* in all three of these scarab species (Koppenhöfer et al., 2000a, 2002). However, considering the high efficacy of *S. scarabaei* alone against white grubs, a combination with imidacloprid appears of limited usefulness.

In summary, *S. scarabaei* shows exceptional potential as a curative control agent for white grubs because of its superiority in efficacy and speed of kill, and the wider range of white grub species susceptible to it compared to other nematode species. In addition, its high degree of adaptation to white grubs makes it a promising candidate for the development of long term white grub control strategies such as augmentative or inoculative releases.

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