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Long-term effects and persistence of *Steinernema scarabaei* applied for suppression of *Anomala orientalis* (Coleoptera: Scarabaeidae)

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ABSTRACT

The entomopathogenic nematode, *Steinernema scarabaei*, is adapted to scarab larvae as hosts and has already shown exceptional potential for inundative control of these pests. To determine the long-term effects of *S. scarabaei* application on scarab populations and the nematode's persistence, *S. scarabaei* was applied in mid-September at rates from 0.06 to 2.5 × 10^9 infective juveniles (IJs)/ha to turfgrass plots seeded with oriental beetle, *Anomala orientalis*, larvae. Scarab and nematode populations were monitored for 3–4 years thereafter. *S. scarabaei* provided excellent *A. orientalis* control (77–100%) within 1 month of application at rates of 0.25–2.5 × 10^9 (IJs)/ha and particularly in the following spring at rates of 0.1–2.5 × 10^9 (IJs)/ha (86–100%). *S. scarabaei* provided significant control in the next *A. orientalis* generation in two out of 10 treatments in fall (i.e., 13 months after application) and six out of 10 treatments in the following spring. Thereafter, significant control was only observed occasionally. *S. scarabaei* numbers were highly variable, and few significant differences among treatments were observed. *S. scarabaei* recovery from the treated plots was generally more consistent through the first spring after application and became more variable thereafter, but *S. scarabaei* was recovered for up to 4 years in the experimental plots. Endemic populations of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*, regularly recovered from the experimental plots and often in higher numbers than *S. scarabaei*, had no significant effect on *A. orientalis* densities but were able to coexist with *S. scarabaei*. Our observations suggest that, once current problems with its mass production can be overcome, *S. scarabaei* could be augmented periodically in areas with recurrent scarab infestations to provide long-term suppression.

1. Introduction

Entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) have been studied extensively for biological control of numerous insect pests in a wide range of commodities (Grewal et al., 2005a). In the vast majority of these studies, nematodes have been used as biopesticides for short-term inundative control. However, compared with synthetic insecticides, nematodes are generally more expensive and have shorter shelf-life and lower stability, and their effective use requires more knowledge and care (Shapiro-Ilan et al., 2002). Consequently, nematode use has been restricted to niche markets due to competition from synthetic insecticides. While the continued pressure from legislation [e.g., Food Quality Protection Act of 1996 (Anonymous, 1996)] and the general public to reduce the use of hazardous insecticides should favor the use of nematodes, the discovery of new 'reduced risk' insecticides will continue to limit a wider adoption of nematodes. Short of major advances in nematode production and formulation technology, a more promising future for entomopathogenic nematodes may lie in developing alternative approaches to their use as biopesticides. In particular, longer-term strategies such inoculative releases, especially in combination with conservation biological control, might ultimately be more cost effective and practical (Lewis et al., 1998).

Kaya (1990) proposed that inoculative applications of entomopathogenic nematodes are more likely to succeed if the pest or pest complex is present throughout much of the year, has a high economic threshold, and is moderately susceptible to nematodes, and if soil conditions are favorable for nematode persistence. Campbell et al. (1995) pointed out that scarab larvae in turfgrass should be an ideal pest and ecosystem for inoculative nematode releases. In the northeastern USA, scarab larvae are present in turfgrass throughout much of the year, their action threshold is relatively high [50–100 larvae/m^2 (Potter, 1998; Vittum et al., 1999)], several of the important scarab species are moderately susceptible to several nematode species (Grewal et al., 2005b), and the turfgrass ecosystem is a relatively stable habitat in which numerous pest and non-pest insect species occur (Potter, 1998; Vittum et al., 1999).

In New Jersey, southeastern New York, Connecticut, and Rhode Island, the oriental beetle, *Anomala* (=*Exomala*) *orientalis*

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Waterhouse, is the dominant scarab species in turfgrass (Alm et al., 1999; AMK, personal observations), but the Japanese beetle, *Popillia japonica* Newman, northern masked chafer, *Cyclocephala borealis* Arrow, and European chafer, *Rhizotrogus majalis* (Razoumowsky), can also occur in high densities (AMK, unpublished data). These species have a similar one-year life cycle (Potter, 1998; Vittum et al., 1999; AMK, personal observation). At the latitude of New Jersey, the adults fly between early June and mid-August. After mating, the females lay eggs among the roots of host plants, and the eggs hatch in 2–3 weeks. The first and second instar each lasts around 3 weeks, so that by mid-September the majority of the larvae are in the third instar. The extensive root-feeding by the larger larvae can kill large areas of grass from mid-August into mid-October, especially under warm dry conditions. In addition, vertebrate predators can tear up the turf to feed on the larvae. After mid-October the larvae overwinter at typically >10 cm soil depth. Damage is less common in the spring when third instars resume feeding near the soil surface until pupation.

Root-feeding scarab larvae, also referred to as white grubs, are parasitized by a large number of entomopathogenic nematode species (Peters, 1996) and several nematode species were originally isolated from white grubs [i.e., *Heterorhabditis megidis* Poinar, Jackson and Klein, *Steinernema anomali* (Kozodoi), *Steinernema glaseri* (Steiner), *Steinernema kushidai* Mamiya, *S. scarabaei* Stock and Koppenhöfer] (Poinar, 1992; Peters, 1996; Stock and Koppenhöfer, 2003). Not surprisingly, nematodes have been studied extensively as biocontrol agents for white grubs (Klein, 1993; Grewal et al., 2005b). However, as for insects in general, nematodes have been used almost exclusively as biopesticides against white grubs. Nevertheless, Klein and Georgis (1992) observed *P. japonica* control by *Heterorhabditis bacteriophora* Poinar into the following generation (i.e., 1 year after application) and suspected that such long-term effects may not be uncommon. Campbell et al. (1995) found that *P. japonica* population densities were about 50% lower in patches where its larvae overlapped with endemic *H. bacteriophora*. But endemic *H. bacteriophora* were patchier in distribution than *P. japonica* larvae which probably limited the nematode's impact on the host populations (Campbell et al., 1995). Inundatively released *H. bacteriophora* also quickly returned to a patchy distribution similar to that of endemic *H. bacteriophora* (Campbell et al., 1998) which may be in part related to the generally poor persistence of *H. bacteriophora* (e.g., Baur and Kaya, 2001; Koppenhöfer and Fuzy, 2006, 2007).

Steinernema scarabaei offers many characteristics that may allow it to have a more stable relationship with host populations than *H. bacteriophora*. *S. scarabaei* was isolated from epizootics in *A. orientalis* and *P. japonica* larvae in turfgrass areas in central New Jersey (Stock and Koppenhöfer, 2003) and appears to be closely adapted to white grubs as hosts (Koppenhöfer and Fuzy, 2003b). Compared to *H. bacteriophora*, it is more virulent (Grewal et al., 2005b; Koppenhöfer et al., 2006) and reproduces more consistently in numerous white grub species (Cappaert and Koppenhöfer, 2003; Koppenhöfer and Fuzy, 2003b) but poorly infects and reproduces in other insect groups (Koppenhöfer and Fuzy, 2003b). *S. scarabaei* is similarly infectious to second- and third-instar *P. japonica* and *A. orientalis* (Koppenhöfer and Fuzy, 2004) and has shown high infection and reproduction rates at soil temperatures as low as 15 °C (Koppenhöfer and Fuzy, 2003b). In contrast, *H. bacteriophora* has generally performed poorly below 20 °C (Georgis and Gaugler, 1991), thus limiting efficacy against or recycling in overwintered third instars. Finally, *S. scarabaei* is highly effective and persists for long periods in a wide range of soil types (Koppenhöfer and Fuzy, 2006) and soil moisture conditions (Koppenhöfer and Fuzy, 2007). Our expectations for long-term suppression of white grubs by a scarab-specific nematode are also inspired by the successful use of another host-specific nematode, *Steinernema scapterisci* Nguyen and Smart, as an inoculative control agent against mole crickets in Florida (e.g., Parkman and Smart, 1996). *S. scapterisci* established successfully in pastures and on golf courses and mean mole cricket trap catches declined by up to 98% within 3 years of releases.

Our objective was to determine the short-term and long-term effects of *S. scarabaei* applications on white grub populations and nematode populations in turfgrass. To this end, a range of *S. scarabaei* rates from the recommended field rate (2.5×10^9 infective juveniles (IJs)/ha) (Georgis and Gaugler, 1991; Grewal et al., 2005b) to as low as 1/40th thereof were tested assuming that higher rates would be better for quick short-term control in late summer/early fall but lower rates may be better for long-term establishment by leaving more larvae to be infected in the following spring for better nematode persistence through the summer. To facilitate comparison among experiments, three 3–4 year experiments were conducted in the same area using the same soil type, grass species, and white grub species.

2. Materials and methods

2.1. General methods

Late second- and early third-instar *A. orientalis* were collected in turfgrass areas at the Rutgers University Plant Science Research Farm (Adelphia, NJ). The larvae were kept individually in the cells of 24-well plates in sandy loam, were brought to the laboratory for identification to species using the raster pattern in front of the anal slit (Potter, 1998) and were released into experimental plots within 48 h of collection. Eggs for release into experimental plots were obtained as follows. Spring-collected *A. orientalis* larvae were reared to adults individually in 30-ml plastic cups filled with sandy loam and with perennial ryegrass, *Lolium perenne* L., growing in the cups as a food source. Fifty to 100 adults were kept in clear 4-liter plastic boxes (30 × 17 × 11 cm) filled to height of 5 cm with sifted moist sandy loam. Eggs were collected weekly by searching through the soil in the boxes. Freshly laid eggs are oblong but absorb water from the soil and become spherical within a few days. To reduce experimental variability in development and hatch rate, only eggs that were already swollen were used for experiments. *S. scarabaei* (AMK001 strain) was cultured in *P. japonica* and *A. orientalis* larvae because its production in wax moth larvae is unreliable (Koppenhöfer, unpublished data). The emerging IJs were harvested over a period of 10 days from emergence traps, i.e., modified White traps (Kaya and Stock, 1997), and stored in tap water at 10 °C for 5–14 days before use.

2.2. Field experiments

Three field experiments were conducted at the Rutgers University Plant Science Research Farm in turfgrass areas planted with Kentucky bluegrass (*Poa pratensis* L.). At the start of the experiments, the areas were 2–4 years old and had thatch layers varying from 4 to 9 mm in thickness. None of the areas had been treated with insecticides since establishment. The areas were maintained using typical management procedures for intermediate turfgrass maintenance levels. Thus, the mowing height was 38 mm, the areas were fertilized as necessary about once a year, and during the growing season the areas received overhead irrigation as necessary to limit drought stress (i.e., about 25 mm per week in the absence of rainfall). The soils were sandy loams (62–69% sand, 18–22% silt, 13–17% clay; 1.7–2.5% OM, pH 6.2–6.7).

For each experimental area, white grub and nematode populations were determined in early September before treatment application. White grub populations were determined by taking 20 sod/soil cores with a golf hole cutter (10.8 cm diam × 10 cm depth) in a grid pattern throughout the experimental area and counting the

number of white grubs in the cores. White grub species was determined using the raster pattern in front of the anal slit (Potter, 1998). Entomopathogenic nematode populations were determined by baiting soil samples with wax moth larvae. From each quadrant of the experimental area four soil cores (2.5 cm diam × 10 cm depth) were taken with an Oakfield sampler and the soil pooled by quadrant and mixed. For each quadrant two 200-ml plastic cups were filled with 100 g moist soil each and five wax moth larvae added. Nematode infection of the larvae was determined 7 days later and nematode species determined based on the color of the host cadaver and morphology of the nematodes found in dissected cadavers (Kaya and Stock, 1997; Koppenhöfer, 2007).

About 1 week before treatments were applied experimental arenas were established consisting of 122 × 122 cm turf squares enclosed by 12.5 cm high plastic barriers (Emerald Edge, Easy Gardener, Waco, TX) pushed into the soil to a depth of about 12 cm. Replicates were arranged in rows with 91.5 cm spacing between replicates in both directions. Three to four days later each replicate received enough field-collected late second- and early third-instar *A. orientalis* to bring the larval density to 10 per 0.1 m^2 including the resident *A. orientalis* populations as determined in the golf hole cutter cores taken before. This density was chosen as it is commonly regarded as the treatment threshold for white grubs in turfgrass (Potter, 1998) and is commonly encountered in the field (Koppenhöfer, personal observations). Released larvae that did not enter the soil within 1 h were replaced. Four days later treatments were applied as a drench in 5 mm water followed by 5 mm irrigation, both applied using a watering can. Treatments were arranged in a randomized complete block design with four or five replicates per treatment.

Following treatment application white grub and nematode populations were determined periodically (Table 1). Sampling equipment for white grubs and for nematodes was wiped clean with a paper towel and ethanol between plots. White grub populations were determined by searching through eight sod/soil cores per plot (two per quadrant) taken with a golf hole cutter and larvae were identified to species using the raster pattern. After examination the larvae, whether alive or dead, were placed back along with the soil and sod.

Nematode populations were determined by saturation baiting of soil samples (Koppenhöfer et al., 1998). Eight sod/soil cores were taken from each plot (two per quadrant) with an Oakfield sampler (2.5 cm diam × 10 cm depth), pooled by plot into plastic bags, and brought to the laboratory in a cooler. The samples were broken up and two 100-g subsamples per plot were filled into petri dishes (90 mm diam × 25 mm height). Five wax moth larvae were added per dish. Every 3 days, dead larvae were replaced with new larvae and baiting in each dish continued until no more infected larvae were found for two consecutive 3-day periods. Dead larvae with signs of nematode infection were dissected and digested in a pepsin solution to count the nematodes established in them (Mauléon et al., 1993). Nematode species were determined by the color of the wax moth cadaver (gray-brown with a hint of green for *S. scarabaei*, orange-red for *H. bacteriophora*, beige for *Steinernema carpocapsae*) and the morphology of nematodes found in the dissected larvae (Kaya and Stock, 1997; Koppenhöfer, 2007).

Sampling dates were selected based on the following reasons. Generally, white grub populations were not determined as often as nematode populations because too frequent sampling would have been very disruptive due to the relatively large area sampled. Nematode establishment in the soil was determined immediately after *S. scarabaei* application. At this time white grub populations were not determined because we assumed that little mortality had occurred in the 4 days since release. White grub and nematode populations were determined in mid- to late October because we assumed that due to decreasing soil temperatures, no additional infection would occur after this time. In early April, before soil temperatures would rise high enough to allow for nematode activity, only nematode populations were sampled to determine their overwintering capabilities. Both white grub and nematode populations were sampled in late May to determine if *S. scarabaei* had any effect on white grub populations during the short period in spring when soil temperature were high enough for nematode activity and before the white grubs pupated. Once white grubs purge their intestines in preparation for pupation, become prepupae, and finally pupate, they become less and less susceptible to *H. bacteriophora* and particularly *S. scarabaei* (Koppenhöfer and Fuzy, 2004). In early to mid-August nematode populations only were sampled to

Table 1
Summary of sampling dates for three field experiments started in September of three consecutive years (2002–2004)

Date	Organism sampled		Days after treatment samples were taken		
	Nematodes	White grubs	Experiment 1	Experiment 2	Experiment 3
Sep 17, 2002	+	–	0	–	–
Oct 18, 2002	+	+	31	–	–
Apr 17, 2003	+	+	211	–	–
Aug 7, 2003	+	–	323	–	–
Sep 19, 2003	+	–	–	0	–
Oct 23, 2003	+	+	399	34	–
Apr 7, 2004	+	–	567	201	–
May 20, 2004	+	+	610	244	–
Aug 13, 2004	+	–	695	329	–
Sep 16, 2004	+	–	–	–	0
Oct 17, 2004	+	+	760	394	31
Apr 5, 2005	+	–	930	564	201
May 23, 2005	+	+	978	612	249
Aug 9, 2005	+	–	1056	690	327
Oct 18, 2005	+	+	1120	760	397
Apr 11, 2006	+	–	1295	935	573
May 24, 2006	+	+	1338	978	616
Aug 4, 2006	+	–	1411	1051	689
Oct 22, 2006	+	+	1490	1130	768
Apr 15, 2007	+	–	–	1299	937
May 24, 2007	+	+	–	1343	981
Aug 10, 2007	+	–	–	1421	1059
Oct 20, 2007	+	+	–	1492	1130

'+' and '–' indicate whether samples were taken or not on the respective date.

determine how well the scarab-specific *S. scarabaei* would persist during the warm summer period when no white grub hosts were available. Finally, grub and nematode populations were again sampled in October to determine any effects of *S. scarabaei* on the grub populations as would be indicated by a decrease in grub density and an increase in *S. scarabaei* densities. Sampling continued following this pattern until 3–4 years after *S. scarabaei* application.

To ensure that at least low *A. orientalis* populations would be present in the plots in the years following treatment applications, *A. orientalis* eggs obtained from the laboratory rearing were implanted into each replicate during each July of the experimental period. Six golf hole cutter cores were taken per plot, the soil at the bottom of the hole loosened, 20 eggs placed into each hole, and the sod/soil core carefully placed back on top. Under optimal laboratory conditions, these 120 eggs per plot (7.5 per 0.1 m^2) would result in about 50 second instars and ultimately in about 25 third instars (Koppenhöfer, unpublished data).

The first experiment was started in September 2002. Preapplication revealed low populations of endemic white grubs (1.2 per 0.1 m^2, 100% *A. orientalis*) and entomopathogenic nematodes (one out of 40 wax moth larvae infected with *H. bacteriophora*). Treatments replicated four times were applied on September 17, 2002 (soil temperature at 5 cm depth 22 °C, air temperature 24 °C, cloudy) consisting of 0, 0.4×10^9, 1.0×10^9, and 2.5×10^9 *S. scarabaei*/ha. Sampling dates are summarized in Table 1.

The second experiment was started in September 2003. Preapplication revealed low populations of endemic white grubs (3.2 per 0.1 m^2, 100% *A. orientalis*) and entomopathogenic nematodes (two out of 40 wax moth larvae infected with *H. bacteriophora*). Treatments replicated four times were applied on September 19, 2003 (soil temperature at 5 cm depth 22 °C, air temperature 24 °C, cloudy) consisting of 0, 0.06×10^9, 0.12×10^9, 0.24×10^9, and 0.6×10^9 *S. scarabaei*/ha. Sampling dates are summarized in Table 1.

The third experiment was started in September 2004. Preapplication revealed low populations of endemic white grubs (1.2 per 0.1 m^2, 100% *A. orientalis*) and entomopathogenic nematodes (two out of 40 wax moth larvae infected with *H. bacteriophora*). Treatments replicated five times were applied on September 16, 2004 (soil temperature at 5 cm depth 21 °C, air temperature 23 °C, cloudy) consisting of 0, 0.1×10^9, 0.25×10^9, and 0.625×10^9 *S. scarabaei*/ha. Sampling dates are summarized in Table 1.

2.3. Statistical analysis

The number of *A. orientalis* larvae per plot were square root-transformed and the number of nematodes (by species) baited from the soil samples (number from two dishes combined) were log-transformed to normalize the data and analyzed using repeated measures ANOVA and Tukey test for means separation (SAS Institute Inc., 2002). Differences among means were considered significant at $P < 0.05$. Means ± SE are presented.

3. Results

3.1. Experiment 1 (2002) (Fig. 1)

Anomala orientalis recovery was significantly affected by *S. scarabaei* application rate ($F = 21.60$; df = 3,143; $P < 0.001$) and sampling date ($F = 8.77$; df = 8,143; $P < 0.001$), but there was a significant interaction between application rate and sampling date ($F = 3.02$; df = 24,143; $P < 0.001$). Recovery was significantly affected by *S. scarabaei* treatment within each sampling date until 978 days after treatment (DAT) (Fig. 1). At 31 DAT, *A. orientalis* recovery in the control plots (8.8 ± 2.2 live larvae per 0.1 m^2, one out of 29 larvae recovered infected by an endemic *H. bacteriophora*) was significantly higher than in all *S. scarabaei*-treated plots. In the treated plots all recovered larvae were infected (28 by *S. scarabaei*, one by *H. bacteriophora*) but the number of *S. scarabaei*-infected larvae varied from 0 to 6 per individual plot and from 1.3 ± 0.5 per plot at the lowest *S. scarabaei* rate to 3.3 ± 0.9 per plot at the intermediate rate without significant differences among treatments. On all following sampling dates, recovery of *S. scarabaei*-infected larvae was much lower with never more than two larvae per individual plot, and never more than five larvae in the entire experiment combined.

When comparing treatment effects within each sampling date, the highest *S. scarabaei* rate caused significant reductions in *A. orientalis* numbers at 31 DAT (100%), 211 DAT (100%), 610 DAT (94%), and 760 DAT (94%), but not on the remaining dates (33–100%); the middle *S. scarabaei* rate caused significant reductions at 31 DAT (100%) and 211 DAT (100%) but not on the remaining dates (35–71%); and the lowest *S. scarabaei* rates caused significant reductions at 31 DAT (100%), 211 DAT (100%), 399 DAT (92%), and 978 DAT (83%) but not on the remaining dates (25–75%). During the fourth year (1338 and 1490 DAT), *A. orientalis* numbers in the control were low, and on none of the sampling dates did *A. orientalis* recovery differ among the different *S. scarabaei* rates. Recovery of *P. japonica* (total of 4) and *C. borealis* (total of 2) larvae was too low and erratic to be included in the analysis.

Steinernema scarabaei numbers extracted from soil samples were significantly affected by *S. scarabaei* application rate ($F = 9.28$; df = 3,192; $P < 0.002$) and sampling date ($F = 8.19$; df = 16,192; $P < 0.001$), but there was a significant interactions between application rate and sampling date ($F = 2.03$; df = 48,192; $P < 0.001$). At 0 DAT, *S. scarabaei* numbers corresponded to the *S. scarabaei* application rates with significantly more *S. scarabaei* at the highest rate (2.5×10^9 IJs/ha) than in the control (0.0), whereas numbers from the two other treatments did not differ significantly from either control or highest rate (Fig. 1). At 31 DAT, significantly more *S. scarabaei* were extracted from all *S. scarabaei* treatments than from the control (0.0) with no significant differences among application rates. Compared to the 0 DAT numbers, *S. scarabaei* numbers had increased 3.8-fold in the lowest and 5.4-fold in the middle *S. scarabaei* rate, but had not changed at the highest rate. At 211 DAT, significantly more *S. scarabaei* were extracted from the plots treated with the middle *S. scarabaei* rate than from control (0.0) whereas numbers from the two other treatments did not differ significantly from either control or middle rate. Compared to the 31 DAT numbers, *S. scarabaei* numbers had remained at the same level in the lowest and middle rates but had declined by 60% in the highest rate.

From 323 DAT onwards, no more significant differences in *S. scarabaei* numbers were observed among treatments. *S. scarabaei* numbers had dropped dramatically in all *S. scarabaei* treatments by 323 DAT (range of means 7.0–18.1) and had further declined by 399 DAT (range of means 1.0–6.8) and 567 DAT (range of means 0.3–2.0). From 610 DAT until the end of the experiment (1490 DAT), *S. scarabaei* numbers showed great fluctuation and variability within treatments usually caused by very high densities (up to 285 IJs/200 g sample) in individual plots. *S. scarabaei* was detected in every treated plot until 323 DAT. Thereafter, it was usually not found in every treated plot with detection rates across all treated plots varying from 33% to 92%.

Steinernema scarabaei was for the first time detected in the control plots at 323 DAT (1.5 ± 0.7), then again at 695 DAT (1.0 ± 0.6), and was consistently recovered (up to 5.0 ± 4.4, but never more than in 2 out of 4 plots) on all sampling dates (except 1411 DAT) starting at 978 DAT until the end of the experiment.

Baiting the soil samples with *G. mellonella* larvae also revealed the presence of endemic populations of *H. bacteriophora* and *S. carpocapsae* which often were found in the majority of plots (*H. bacteriophora*: average 13.5 out of 16, range 9–16; *S. carpocap-*

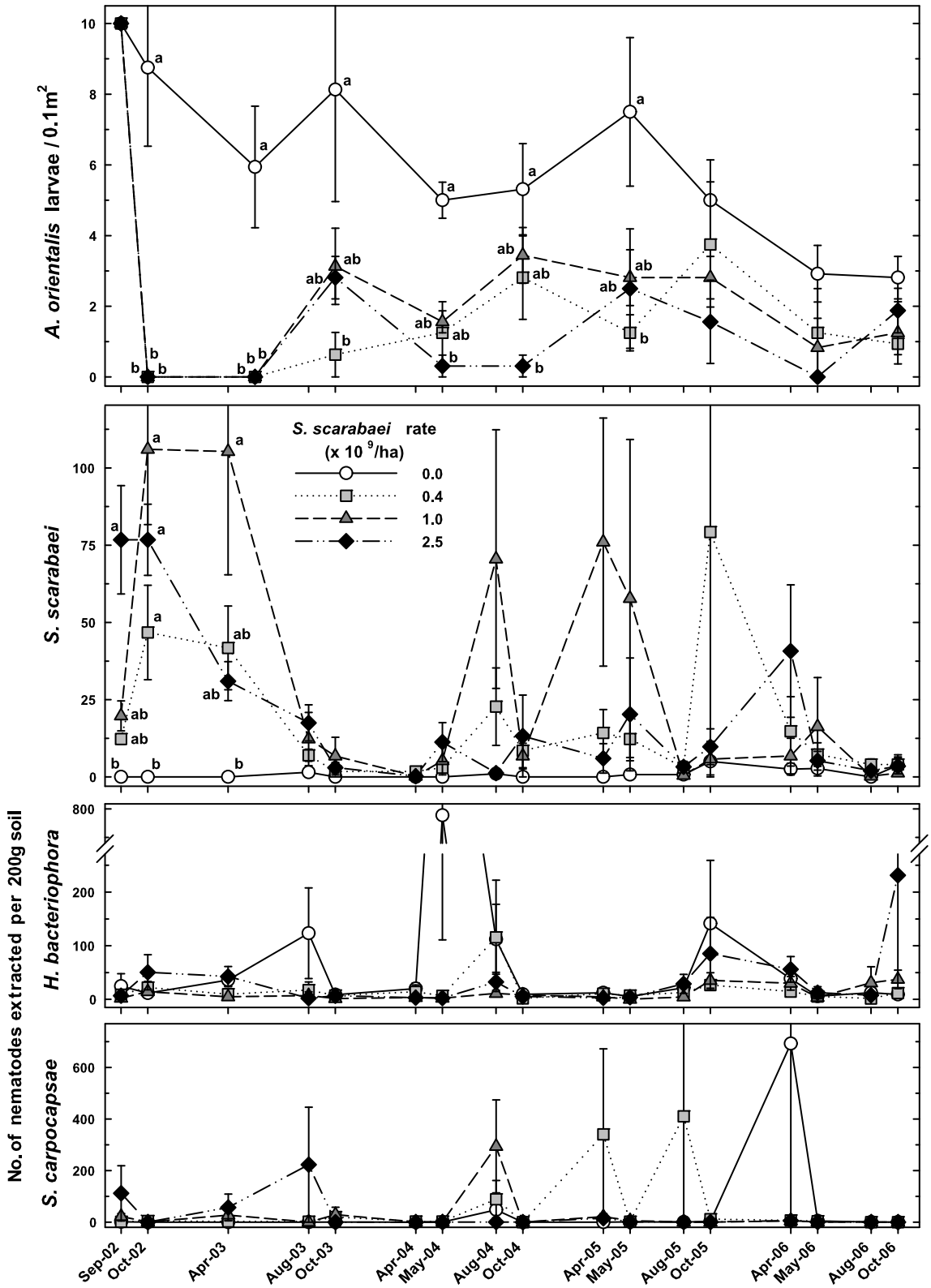

Fig. 1. Population densities (±SE) of larval *Anomala orientalis* and the entomopathogenic nematodes *Steinernema scarabaei*, *Heterorhabditis bacteriophora*, and *S. carpocapsae* after application (mid-September 2002) of different *S. scarabaei* rates to turfgrass microplots seeded with late second- and early third-instar *A. orientalis* (10 per 0.1 m^2). Means with the same letter within sampling date are not significantly different; no letters are shown for dates without significant differences among means ($P < 0.05$, Tukey).

sae: average 5.4 out of 16, range 0–11), and often in higher numbers than *S. scarabaei* (Fig. 1). While densities of both species significantly varied with sampling date ($F \geqslant 4.83$; df = 16,192; $P < 0.001$), they were not affected by *S. scarabaei* application rate and there was no interaction between application rate and sampling date.

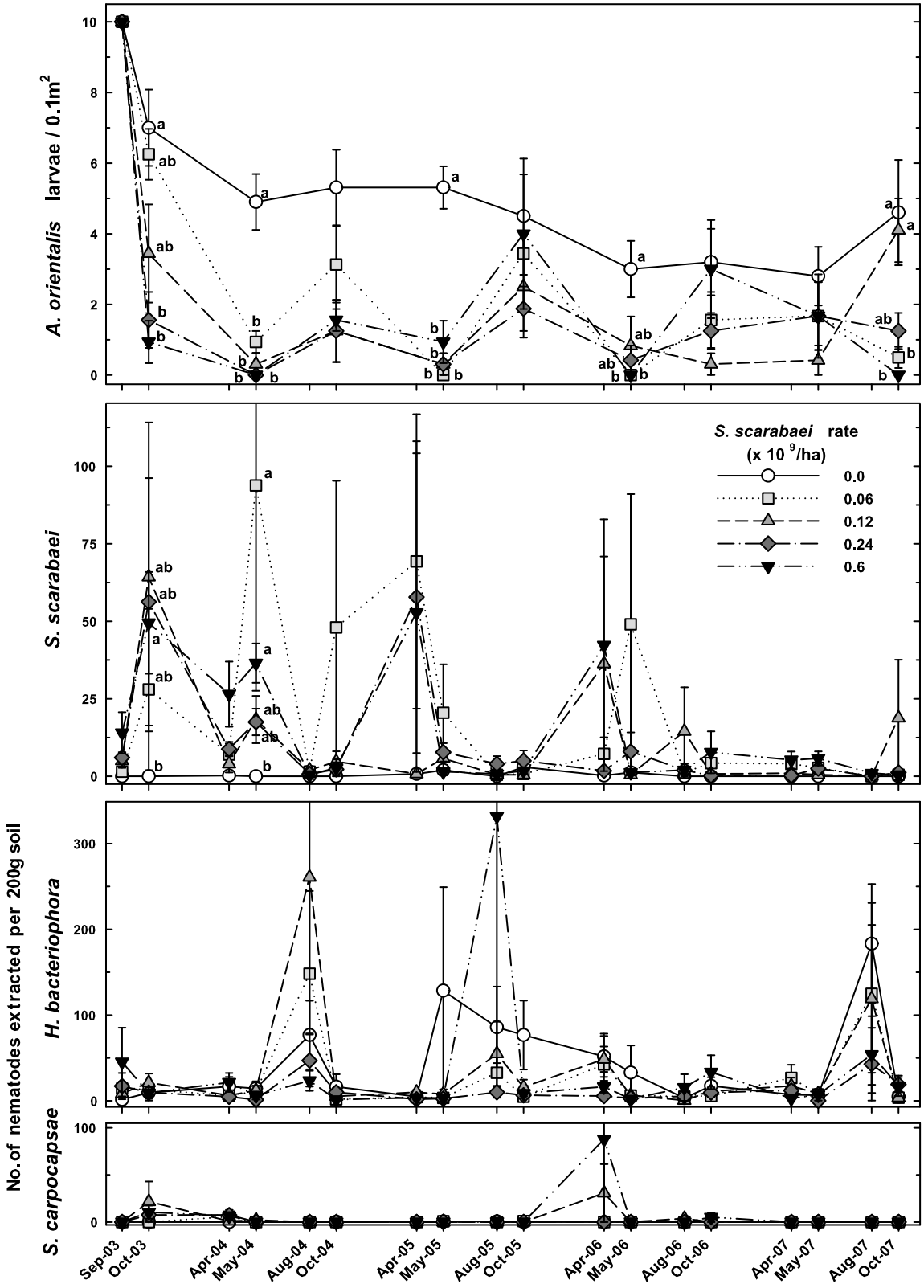

Fig. 2. Population densities (±SE) of larval *Anomala orientalis* and the entomopathogenic nematodes *Steinernema scarabaei*, *Heterorhabditis bacteriophora*, and *S. carpocapsae* after application (mid-September 2003) of different *S. scarabaei* rates to turfgrass microplots seeded with late second- and early third-instar *A. orientalis* (10 per 0.1 m^2). Means with the same letter within sampling date are not significantly different; no letters are shown for dates without significant differences among means ($P < 0.05$, Tukey).

3.2. Experiment 2 (2003) (Fig. 2)

Anomala orientalis recovery was significantly affected by *S. scarabaei* application rate ($F = 24.16$; df = 4,179; $P < 0.001$) and sampling date ($F = 9.43$; df = 8,179; $P < 0.001$), but there was a significant interaction between application rate and sampling date ($F = 2.61$; df = 32,179; $P < 0.001$). At 31 DAT no infected larvae were found in the control. The number of *S. scarabaei*-infected

larvae in the treated plots varied from 0 to 4 per individual plot and from 0.5 ± 0.3 at the highest *S. scarabaei* rate to 1.3 ± 1.0 at the lowest rate without significant differences among rates. On all following sampling dates, recovery of *S. scarabaei*-infected larvae was much lower with never more than one larva found in a plot and never more than five larvae in the entire experiment combined. No infections by other nematode species were found.

When comparing treatment effects within each sampling date, *A. orientalis* recovery was significantly affected by application rate mostly on the spring sampling dates (Fig. 1). The highest *S. scarabaei* rate caused significant reductions in *A. orientalis* numbers at 34 DAT (87%), 244 DAT (100%), 612 DAT (82%), 978 DAT (100%), and 1492 DAT (100%) but not on the remaining dates (5–71%); the second highest rate caused significant reductions at 34 DAT (77%), 244 DAT (100%), and 612 DAT (94%), but not on the remaining dates (33–77%); the third highest rate caused significant reductions at 244 DAT (93%) and 612 DAT (94%), but not on the remaining dates (5–89%); and the lowest rate caused significant reductions at 244 DAT (80%), 612 DAT (100%), 978 DAT (100%), and 1492 DAT (86%), but not on the remaining dates (9–45%). From 760 DAT until the end of the experiment, *A. orientalis* numbers in the control remained relatively low, making detection of significant treatment effects more challenging. On none of the sampling dates did *A. orientalis* recovery differ among the different *S. scarabaei* rates. Recovery of *P. japonica* (total of 5) and *C. borealis* (total of 3) larvae was too low and erratic to be included in the analysis.

Steinernema scarabaei numbers extracted from soil samples were significantly affected by *S. scarabaei* application rate ($F = 7.73$; df = 4,359; $P < 0.002$) and sampling date ($F = 7.18$; df = 17,359; $P < 0.001$), but there was a significant interactions between application rate and sampling date ($F = 1.42$; df = 68,359; $P < 0.05$). At 0 DAT, *S. scarabaei* numbers did not differ significantly between the control (0.0) and the treatments (2–14). At 34 DAT, significantly more ($F = 3.45$; df = 4,12; $P < 0.05$) *S. scarabaei* were extracted from the highest *S. scarabaei* rate (49.5 ± 16.4) than from control plots (0.0), with the remaining rates not significantly different from control and highest rate (range of means 28.0–64.2 *S. scarabaei*). Compared to 0 DAT *S. scarabaei* numbers in the *S. scarabaei* treatments had increased 3.5- to 18.7-fold. At 201 DAT, *S. scarabaei* numbers were not significantly affected by treatment (4.0 to 26.5 from *S. scarabaei* treatments; 0.3 ± 0.3 in control). At 244 DAT, *S. scarabaei* numbers had increased in all *S. scarabaei* treatments (range of means 17.5–93.8) compared to 201 DAT and were significantly higher than in the control (0.0) in the highest and the lowest *S. scarabaei* treatments.

By 329 DAT, *S. scarabaei* numbers had dropped dramatically in all *S. scarabaei* treatments (range of means 0.0–2.0) and were not significantly different from the control (0.0). From 329 DAT until the end of the experiment (1492 DAT), no significant differences among treatments were detected even though *S. scarabaei* densities varied greatly. During this period *S. scarabaei* numbers were generally low but there were occasional spikes that were mostly caused by very high densities in individual plots. While *S. scarabaei* had been recovered from 88% to 100% of the treated plots until 244 DAT, from 329 DAT onwards, recovery rates were generally lower with 31% to 75% and only 6% at 1421 DAT.

Steinernema scarabaei was detected in the control plots from 564 DAT to 978 DAT but only in one or two of the plots and at very low numbers (0.3–3.0) and was no longer detected thereafter until the end the experiment.

Baiting also revealed the presence of endemic populations of *H. bacteriophora* and *S. carpocapsae*. *H. bacteriophora* was usually recovered from the majority of plots (average 16.9 out of 20, range 8–20) and often in higher numbers than *S. scarabaei* in the treated plots. In contrast, *S. carpocapsae* was mostly recovered in only a few plots (average 1.7 out of 20, range 0–9) and rarely at high numbers. Densities of both species significantly varied with sampling date ($F \geqslant 3.69$; df = 17,359; $P < 0.001$) but were not affected by *S. scarabaei* application rate and there was no interaction between application rate and sampling date.

3.3. Experiment 3 (2004) (Fig. 3)

Anomala orientalis recovery was significantly affected by *S. scarabaei* application rate ($F = 11.43$; df = 3,139; $P < 0.001$) and sampling date ($F = 3.15$; df = 6,139; $P < 0.01$); there was no interaction between application rate and sampling date. All *S. scarabaei* treatments had significantly lower *A. orientalis* recovery than the control without significant differences among rates. Recovery was significantly lower at 616 DAT than at 31 DAT, with no differences among any other sampling dates. At 31 DAT no infected larvae were found in the control. The number of *S. scarabaei*-infected larvae in the treated plots varied from 0 to 2 per individual plot and from 0.0 at the lowest *S. scarabaei* rate to 0.8 ± 0.5 at the intermediate rate without significant differences among rates. On all following sampling dates, recovery of *S. scarabaei*-infected larvae was much lower with up to three larvae in individual plots but never more than four larvae in the entire experiment combined. No infections by other nematode species were found.

When comparing treatment effects within each sampling date, the highest *S. scarabaei* rate caused significant reductions in *A. orientalis* numbers at 31 DAT (93%) and 249 DAT (96%), but not on the remaining dates (55–75%); the second highest rate caused significant reductions at 616 DAT (100%) but not on the remaining dates (55–85%); and the lowest rate caused significant reductions at 31 DAT (82%), 249 DAT (92%), 398 DAT (80%), and 616 DAT (100%), but not on the remaining dates (55–73%). From 398 DAT until the end of the experiment, *A. orientalis* numbers in the control remained relatively low, making detection of significant treatment effects more challenging. On none of these sampling dates did *A. orientalis* recovery differ among treatments. Recovery of *P. japonica* (total of 6) and *C. borealis* (total of 1) larvae was too low and erratic to be included in the analysis.

Steinernema scarabaei numbers extracted from soil samples were significantly affected by *S. scarabaei* application rate ($F = 7.49$; df = 3,279; $P < 0.003$) and sampling date ($F = 6.64$; df = 13,359; $P < 0.0001$), but there was a significant interaction between application rate and sampling date ($F = 1.71$; df = 39,279; $P < 0.01$). *S. scarabaei* numbers differed among treatments at 31 DAT when the highest *S. scarabaei* rates had higher numbers than the lowest rates and the control (Fig. 3). *S. scarabaei* numbers where similar between 31 and 249 DAT, but declined to very low numbers by 328 DAT and remained low until the end the experiment except for two peaks in the second highest *S. scarabaei* rate caused by high numbers in individual plots.

Until 249 DAT, *S. scarabaei* was recovered from 80% to 93% of the treated plots. But from 328 to 981 DAT, recovery from the treated plots ranged from 20% to 47% with only 0% at 1059 DAT and 13% at 1130 DAT. *S. scarabaei* was recovered from the control plots at 0, 201, 328, and 1130 DAT; however, each time only one nematode was detected.

Baiting also revealed the presence of endemic populations of *H. bacteriophora* and *S. carpocapsae*. *H. bacteriophora* was usually recovered from the majority of plots (average 16.8 out of 20, range 11–20) and often in higher numbers than *S. scarabaei* in the treated plots. *S. carpocapsae* was usually recovered in lower numbers and in fewer plots (average 3.5 out of 20, range 0–12). Densities of both species significantly varied with sampling date ($F \geqslant 3.57$; df = 13,279; $P < 0.001$) but were not affected by *S. scarabaei* application rate and there was not interaction between application rate and sampling date.

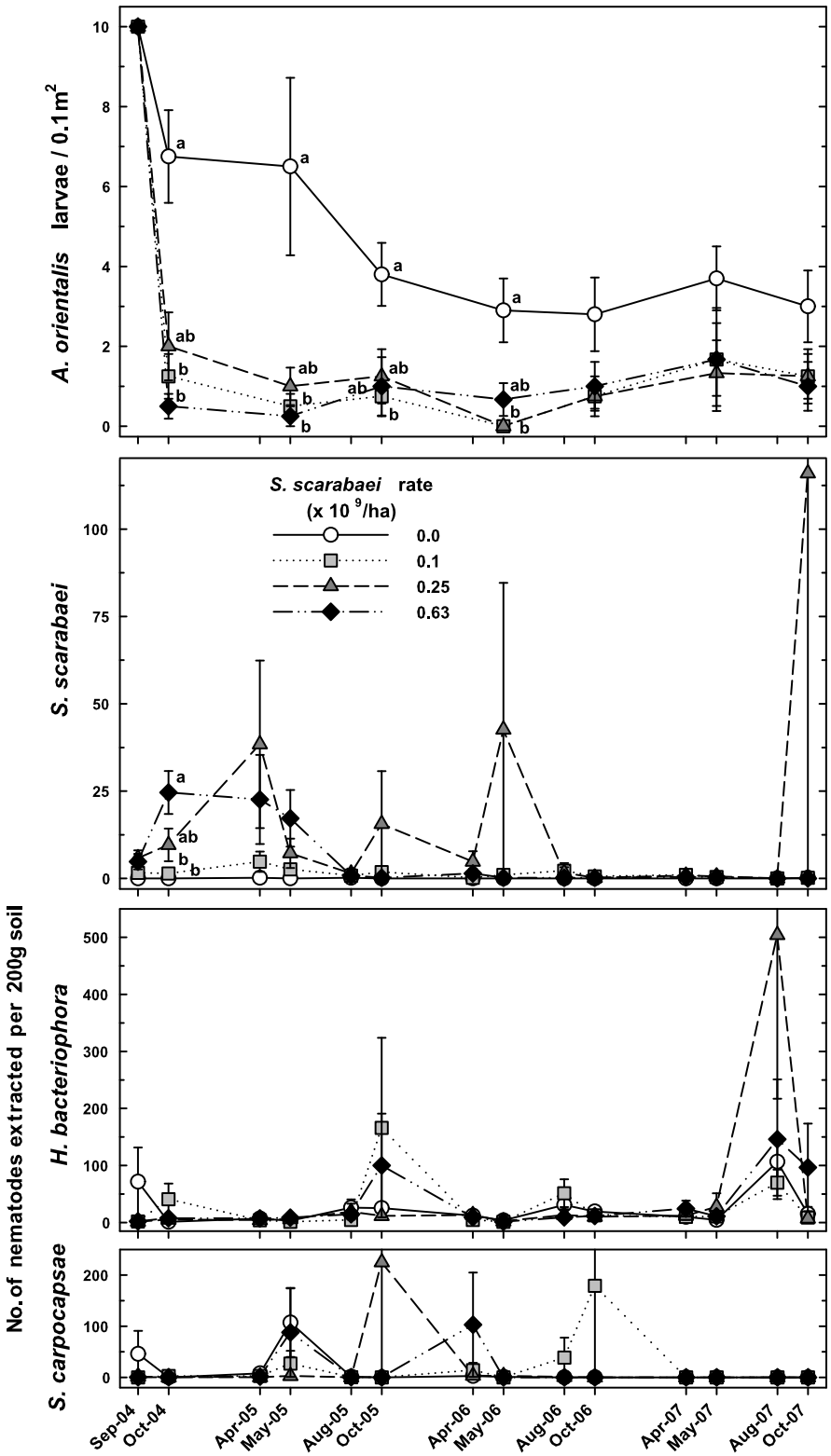

Fig. 3. Population densities (±SE) of larval *Anomala orientalis* and the entomopathogenic nematodes *Steinernema scarabaei*, *Heterorhabditis bacteriophora*, and *S. carpocapsae* after application (mid-September 2004) of different *S. scarabaei* rates to turfgrass microplots seeded with late second- and early third-instar *A. orientalis* (10 per 0.1 m^2). Means with the same letter within sampling date are not significantly different; no letters are shown for dates without significant differences among means ($P < 0.05$, Tukey).

4. Discussion

Steinernema scarabaei provided excellent control of *A. orientalis* larval populations within 1 month of application at very low application rates (0.25 to 2.5 × 10^9 (IJs)/ha) and even more consistent control in the following spring at even lower rates (0.1–2.5 × 10^9 (IJs)/ha). *S. scarabaei* often provided significant control of the following *A. orientalis* generation (i.e., 13 months after application) with again more consistent control in the second spring after application. Thereafter, significant control was only observed

occasionally, but larval densities remained numerically lower in all treatments than in the untreated plots on all sampling dates. During the first year after application, *S. scarabaei* numbers were fairly consistent with strong increases during the first month after application and/or in the following spring. But *S. scarabaei* numbers decreased during the first summer after application and, similar to the *A. orientalis* suppression rates, became more variable over time with less consistent recovery form the plots, presumably due to an increase in patchiness.

The short-term effects of *S. scarabaei* on *A. orientalis* in this study were stronger than in previous studies which should be related to the slightly longer period between application and evaluation. In previous studies evaluated at around 21 DAT, we had observed 60–95% control of *A. orientalis* at rates of $1.0–2.5 \times 10^9$ *S. scarabaei*/ha but only 43% control at 0.4×10^9 *S. scarabaei*/ha (Koppenhöfer and Fuzy, 2003a,c). In our study, rates of 0.25–2.5×10^9 *S. scarabaei*/ha provided 77–100% control at 31–34 DAT. The observed increases in *S. scarabaei* densities between 0 DAT and the first evaluation date indicate that *S. scarabaei* reproduced very well in infected larvae. This is also suggested by the recovery of numerous *S. scarabaei*-infected larvae at the first evaluation. Recovery of infected larvae on later sampling dates was lower since infections were likely to be less synchronized and cadavers can only be recovered for a period of about 10 days after death occurs.

The high control rates compared to the 21 DAT evaluations strongly suggest that *S. scarabaei* progeny emerging from the initially infected larvae provided additional control. Koppenhöfer and Fuzy (2003a) observed a more limited increase in *A. orientalis* control between 21 and 39 DAT (e.g., at 0.4×10^9 *S. scarabaei*/ha control increased from 43% to 63%) which may have been related to relatively dry conditions during the latter part of that experiment. Ongoing studies suggest that applications done 2–3 weeks earlier than in our study could further increase *S. scarabaei* efficacy (Koppenhöfer, unpublished data) which is not surprising because the soil temperatures past late September should increasingly limit *S. scarabaei* activity (Koppenhöfer and Fuzy, 2003b).

The effect of *S. scarabaei* on *A. orientalis* populations did not differ significantly among application rates except in the third experiment at 1492 DAT. At the first evaluation date this was due to the 100% control by all treatments in the first experiment and probably due to the great variability in the data in the second and third experiment. However, at later evaluation dates, there was not even a numerical (i.e., not statistically significant) trend in efficacy of the different rates. This lack in effect of *S. scarabaei* application rate on *A. orientalis* numbers is paralleled by (and probably related to) a similar lack of effect on *S. scarabaei* densities in the soil. Slightly later soil sampling in October and especially in May might have revealed higher *S. scarabaei* densities as that would have allowed nematode progeny to emerge from the *S. scarabaei*-infected *A. orientalis* larvae regularly recovered during those evaluations. However, the downwards movement of larvae in preparation for overwintering in October and pupation in May would have made evaluations for larval densities more difficult.

Two factors may have limited the effect of *S. scarabaei* on *A. orientalis* populations. First, in all three experiments *A. orientalis* populations were never higher than 10 larvae per 0.1 m^2 in the untreated plots. It is possible that higher larval populations may have increased *S. scarabaei* persistence and with that probably also long-term control efficiency as the nematode should have a positive feed-back with its host's densities. Second, after 2–3 years, *S. scarabaei* started to show up in some of the untreated control plots, although not consistently and usually at very low numbers. This contamination may have contributed to the often low larval densities in the control, and with that reduced the relative *A. orientalis* suppression in the treated plots. From an experimental standpoint, the contamination was unfortunate, however, it indicates that the nematode can spread (or be spread in soil/sod particles caught on the tires of turfgrass mowers during wet conditions) and establish new foci of infections.

One major limitation for the effective long-term suppression of white grub populations by *S. scarabaei* appears to be its poor survival during the summer month. *S. scarabaei* densities generally decreased to very low levels between the May and August evaluations, and this must have limited the nematode's impact on the new *A. orientalis* generation arising from the eggs laid in June/July. The summer crash in *S. scarabaei* densities is not surprising for the following reasons. *S. scarabaei* does not infect *A. orientalis* (and *P. japonica*) larvae that have purged their intestine in preparation for pupation, prepupae, and pupae (Koppenhöfer and Fuzy, 2004) and is unlikely to infect adults or eggs. It is not known whether *S. scarabaei* can infect first instar larvae, but progeny production from this small stage would be minimal. Second instars of *A. orientalis* and *P. japonica* are highly susceptible to *S. scarabaei* (Koppenhöfer and Fuzy, 2004) but progeny production from second instars is only about 20% of that from third instars (Koppenhöfer, unpublished data). Finally, *S. scarabaei* poorly infects and poorly reproduces in other typical turfgrass insects such as billbug larvae, sod webworms, and cutworms (Koppenhöfer and Fuzy, 2003b). Thus, IJs produced from infections during fall or spring have to survive into mid-August before significant reproduction may occur. Nevertheless, enough IJ numbers seem to survive the summer to allow suppression of white grubs in late summer/early fall and perpetuate *S. scarabaei* populations, albeit in an increasingly patchy distribution.

Endemic populations of *H. bacteriophora* and *S. carpocapsae* were found in all experiments but the lack of correlation between their numbers and *A. orientalis* numbers indicated that they had no significant impact on *A. orientalis* populations. *S. carpocapsae* is ineffective against white grubs in general, whereas *H. bacteriophora* has shown limited effects on *A. orientalis* after inundative releases (Grewal et al., 2005b). Given the dominance of *A. orientalis* in our plots and the competition from the much more virulent *S. scarabaei*, it was no surprise that in all three experiments combined, only two recovered scarab larvae were infected by *H. bacteriophora* and none by *S. carpocapsae*. The lack of significant effects of *S. scarabaei* applications on recovery of *H. bacteriophora* or *S. carpocapsae* indicates that these species were able to coexist for the duration of our experiments. Both *S. carpocapsae* and *H. bacteriophora* infect a wider range of host species (Peters, 1996; Grewal et al., 2005a,b) and probably persisted primarily on various other turfgrass insects such as larvae of sod webworms, cutworms, and billbugs.

Steinernema carpocapsae and especially *H. bacteriophora* were often recovered in higher numbers from the soil samples and are also more virulent to wax moth larvae than *S. scarabaei* (Koppenhöfer, unpublished data). It is therefore possible that *S. scarabaei* numbers were underestimated in samples containing high numbers of the other two species. However, correlation analysis showed no significant negative effects of the presence of either *S. carpocapsae* or *H. bacteriophora* on *S. scarabaei* numbers.

The recovery rate for *S. scarabaei* in the samples taken directly after application averaged 17%, 18%, and 10% in experiments 1, 2, and 3, respectively. These values were calculated based on the area sampled per plot (39.2 cm^2) and the ratio of soil baited (200 g) to total soil sample (500 g), resulting in the equivalent of 15.7 cm^2 soil surface baited. In laboratory experiments using a similar but pasteurized sandy loam as was found in our field experiments, *S. scarabaei* recovery rates at 0 DAT in moderately moist soil (−10 to −100 kPa soil water potential) were in the 20–31% range. The lower recovery rates from the field experiments were probably due to two factors. Some limited loss of IJs may have occurred due to UV radiation, high temperature, and/or desiccation at the

soil surface (Kaya, 1990) between application and sampling (0.5–2 h). However, since baiting out of the soil samples took about 6 days for the majority of IJs but up to several weeks for some IJs, it is likely that natural enemies of nematodes likely to occur in the field soil (Kaya and Koppenhöfer, 1996) played a significant role in reducing recovery.

In conclusion, *S. scarabaei* has great potential as a biocontrol agent for white grubs, both for short-term control and long-term suppression. Unfortunately, mass production of *S. scarabaei* has thus far proven to be difficult, and solving this bottleneck will require more basic research on the nematode's and its symbiont's nutritional requirements and reproduction biology. Once current problems with mass production of *S. scarabaei* can be overcome, it could be released periodically (e.g., every 2–4 years) in areas with recurrent grub infestations to provide long-term suppression. Due to the relatively low application rates required to establish *S. scarabaei* populations and its long persistence, its use will be not only safer but could also be more economical than that of synthetic insecticides.

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