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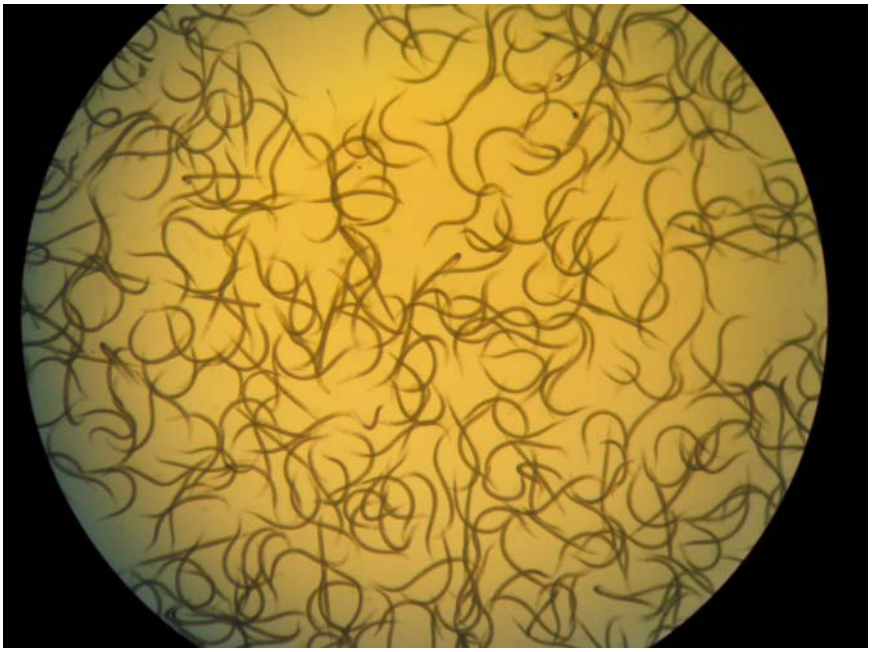

Work continues at Rutgers University to determine the short- and long-term effects of entomopathogenic nematodes for control of white grubs. Their work suggests that once current problems with its mass production can be overcome, *S. scarabaei* could be augmented periodically in areas with recurrent white grub infestations to provide long-term suppression.

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PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 390 projects at a cost of $29 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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Long-term Effects and Persistence of Nematodes for Suppression of White Grubs

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SUMMARY

Steinernema scarabaei has already shown exceptional potential for inundative control of white grubs. We conducted three field experiment to determine the long-term effects of *S. scarabaei* application on white grub 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. White grub and nematode populations were monitored for 3-4 years thereafter. Results include:

- *S. scarabaei* provided 77-100% *A. orientalis* control within 1 month of application at rates of 0.25 to 2.5 × 10^9 IJs/ha and 86-100% control in the following spring at rates of 0.1 to 2.5 × 10^9 IJs/ha.
- *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.
- Recovery of *S. scarabaei* from soil samples was highly variable with few significant differences among treatments 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 *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* populations were regularly recovered from the experimental plots, often in higher numbers than *S. scarabaei*, but had no significant effect on *A. orientalis* population densities.
- Results suggest that once current problems with its mass production can be overcome, *S. scarabaei* could be augmented periodically in areas with recurrent white grub infestations to provide long-term suppression.

Root-feeding white grubs are parasitized by a large number of entomopathogenic nematode species (24, 23, 27). Not surprisingly, nematodes have been studied extensively as biocontrol agents for white grubs (8, 11). However, nematodes have been used almost exclusively as biopesticides. Because nematodes tend to be more expensive and have shorter shelf-life and lower stability than synthetic insecticides, nematode use has been restricted to niche markets. While the continued pressure from legislation (e.g., Food Quality Protection Act of 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 longer-term strategies such as inoculative releases, especially in combination with conservation biological control (21).

Oriental beetle larva infected with *S. scarabaei* showing the typical yellowish color caused by the nematode's symbiotic bacteria.

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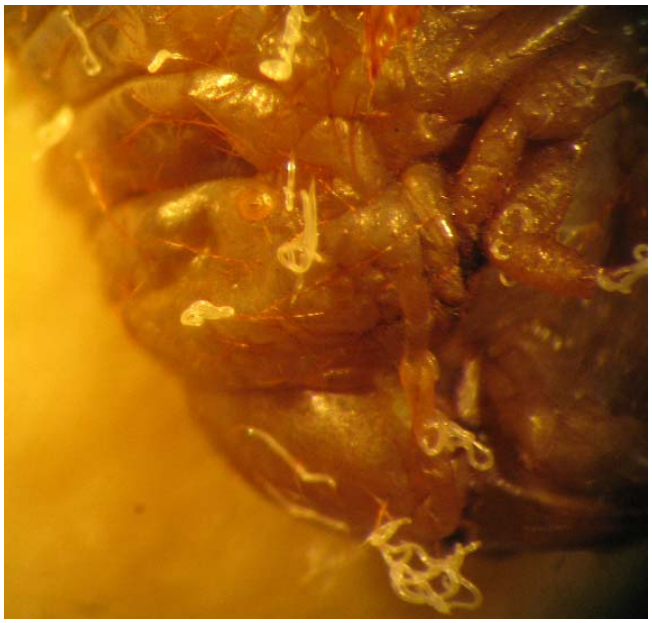


Infective juveniles of *S. scarabaei* emerged from their white grub host.

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 (9). 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 (25, 28)], several of the important scarab species are moderately susceptible to several nematode species (8), and the turfgrass ecosystem is a relatively stable habitat in which numerous pest and non-pest insect species occur (25, 28).

One previous study observed Japanese beetle, *P. japonica*, control by *Heterorhabditis bacteriophora* into the following generation (i.e., 1 year after application) and the authors suspected that such long-term effects may not be uncommon (12). In natural populations, *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. japonic*a larvae which probably limited the nematode's impact on the host populations (3). Inundatively released *H. bacteriophora* also quickly returned to a patchy distribution similar to that of endemic *H. bacteriophora* (4) which may be in part related to the generally poor persistence of *H. bacteriophora* (2, 18, 19).

Steinernema scarabaei for Long-term White Grub Suppression

Steinenema 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 oriental beetle, *A. orientalis,* and *P. japonica* larvae in turfgrass areas in central New Jersey (27) and appears to be closely adapted to white grubs as hosts (15). Compared to *H. bacteriophora*, it is more virulent (8, 20) and reproduces more consistently in numerous white grub species (5, 15) but poorly infects and reproduces in other insect groups (15). *S. scarabaei* is similarly infectious to second- and third-instar *P. japonica* and *A. orientalis* (17) and has shown high infection and reproduction rates at soil temperatures as low as 15 °C (15). In contrast, *H. bacteriophora* has generally performed poorly below 20 °C (6), 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 (18) and soil moisture conditions (19). 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*, as an inoculative control agent against mole crickets in Florida (22). *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) (6, 8) to as low as 1/40th thereof were tested assuming that higher rates would be better for quick short-term

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	+	-	1,056	690	327
Oct 18, 2005	+	+	1,120	760	397
Apr 11, 2006	+	-	1,295	935	573
May 24, 2006	+	+	1,338	978	616
Aug 4, 2006	+	-	1,411	1,051	689
Oct 22, 2006	+	+	1,490	1,130	768
Apr 15, 2007	+	-	-	1,299	937
May 24, 2007	+	+	-	1,343	981
Aug 10, 2007	+	-	-	1,421	1,059
Oct 20, 2007	+	+	-	1,492	1,130

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

Table 1. Summary of sampling dates for three field experiments started in September of three consecutive years (2002, 2003, 2004)

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.

Field Experiment Methods

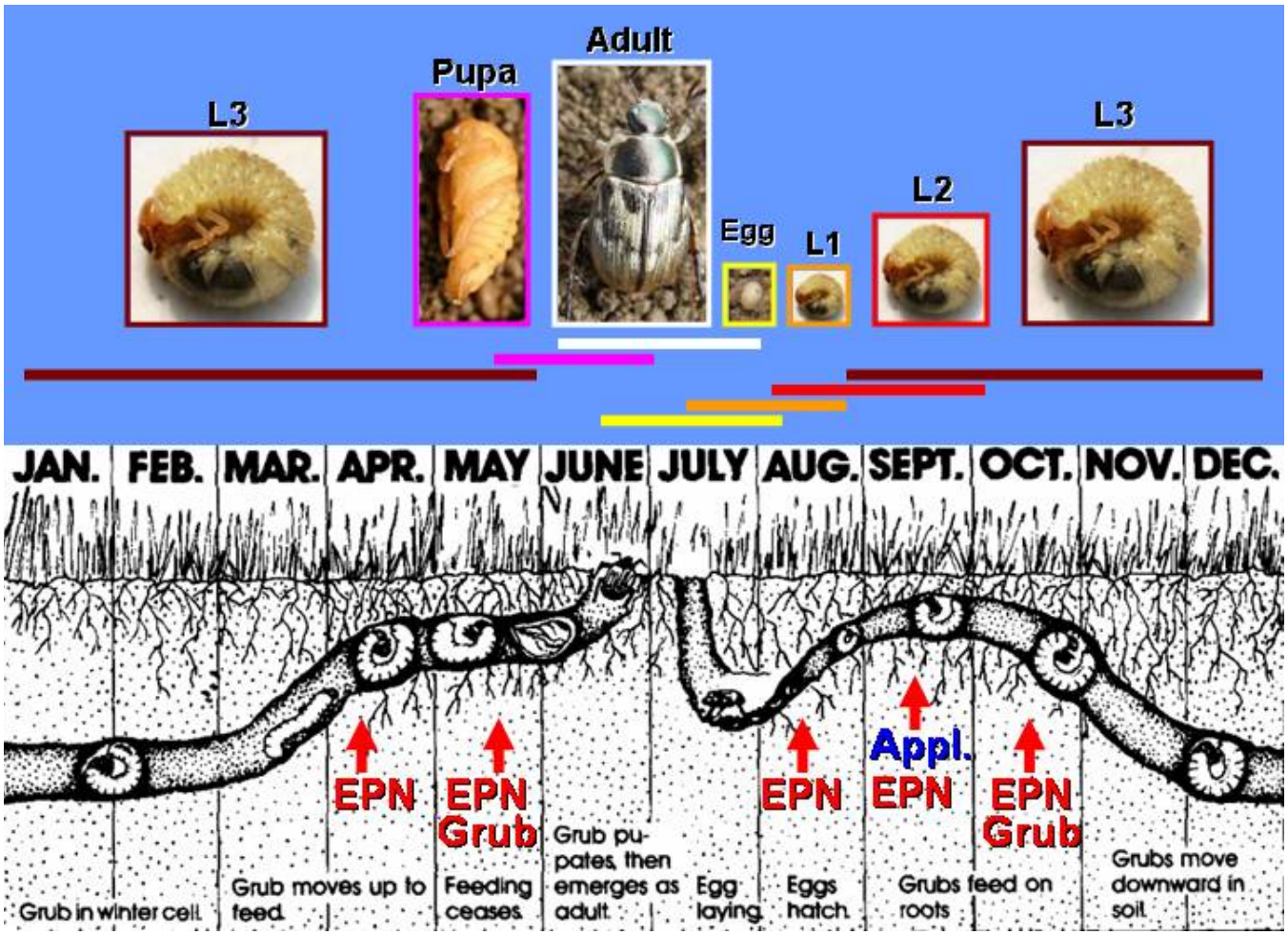
Three field experiments were conducted in Kentucky bluegrass (*Poa pratensis*) area at the Rutgers University Plant Science Research Farm. At the start of the experiments, the areas were 2-4 years old and had thatch layers varying from 4 to 9 mm in thickness. None of the areas had been treated with insecticides since establishment. The areas were mowed at 38 mm height, fertilized as necessary about once a year, and during the growing season 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 (25). Entomopathogenic nematode populations were determined by baiting soil samples with wax moth larvae.

About 1 week before treatment application, experimental arenas were established consisting of 122 cm × 122 cm turf squares enclosed by 12.5 cm high plastic barriers pushed into the soil to a depth of 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. Released larvae that did not enter the soil within 1 hour were replaced. Treatments were applied 4 days later 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. S. *scarabaei* (AMK001 strain) had been cultured in *P. japonica* and *A. orientalis* larvae and stored in tap water at 10 °C for 5-14 days before use.

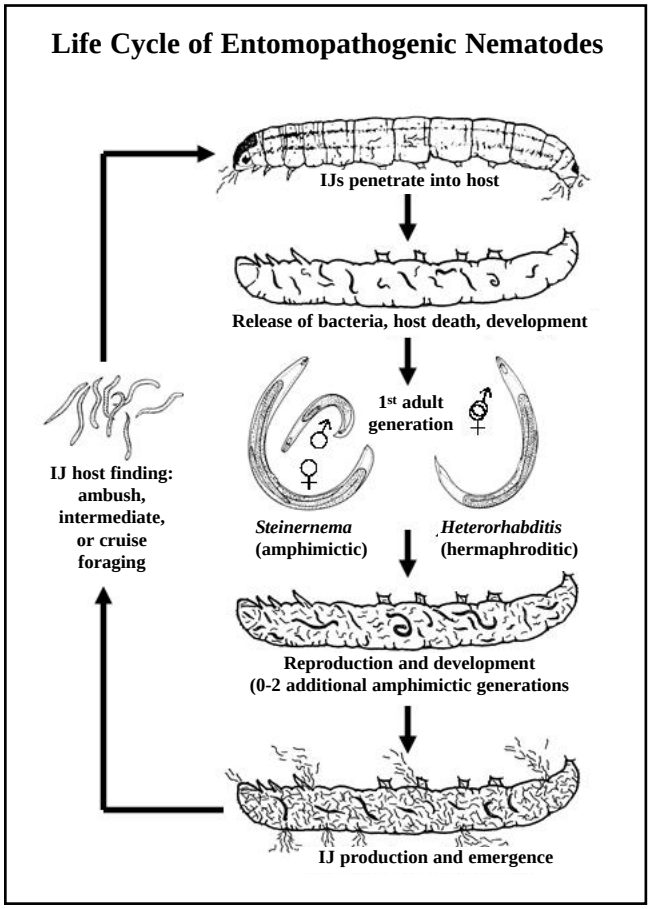
Following treatment application white grub and nematode populations were determined periodically (Table 1). Sampling equipment for white grubs and for nematodes was wiped clean with a paper towel and ethanol between plots. White grub populations were determined by searching through eight sod/soil cores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



Oriental beetle seasonal life-cycle and life stages (top). Arrows indicate periods when entomopathogenic nematodes (EPN) and white grubs were sampled during the study.

raster pattern. After examination, the larvae, alive or dead, were placed back along with the soil and sod.

Nematode populations were determined by saturation baiting of soil samples (13). Eight sod/soil cores (2.5 cm diam × 10 cm depth) were taken from each plot (two per quadrant) and pooled by plot. 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. 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 *S. carpocapsae*) and the morphology of nematodes found in the dissected larvae.



The life cycle of entomopathogenic nematodes includes their penetration into the grub host, release of symbiotic bacteria which kill the grub, reproduction of the nematodes within the host, and the nematodes' emergence from the grub cadaver starting the cycle all over again.

Sampling dates were selected based on the following reasons. 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 late October because we assumed that due to decreasing soil temperatures, no additional infection would occur after this time. In early April, before rising soil temperatures would 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* (17). In early to mid-August nematode populations only were sampled to 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 a laboratory rearing were implanted into each replicate during each July of the experimental period. Six golf hole cutter cores were taken per plot, 20 eggs placed into each hole,

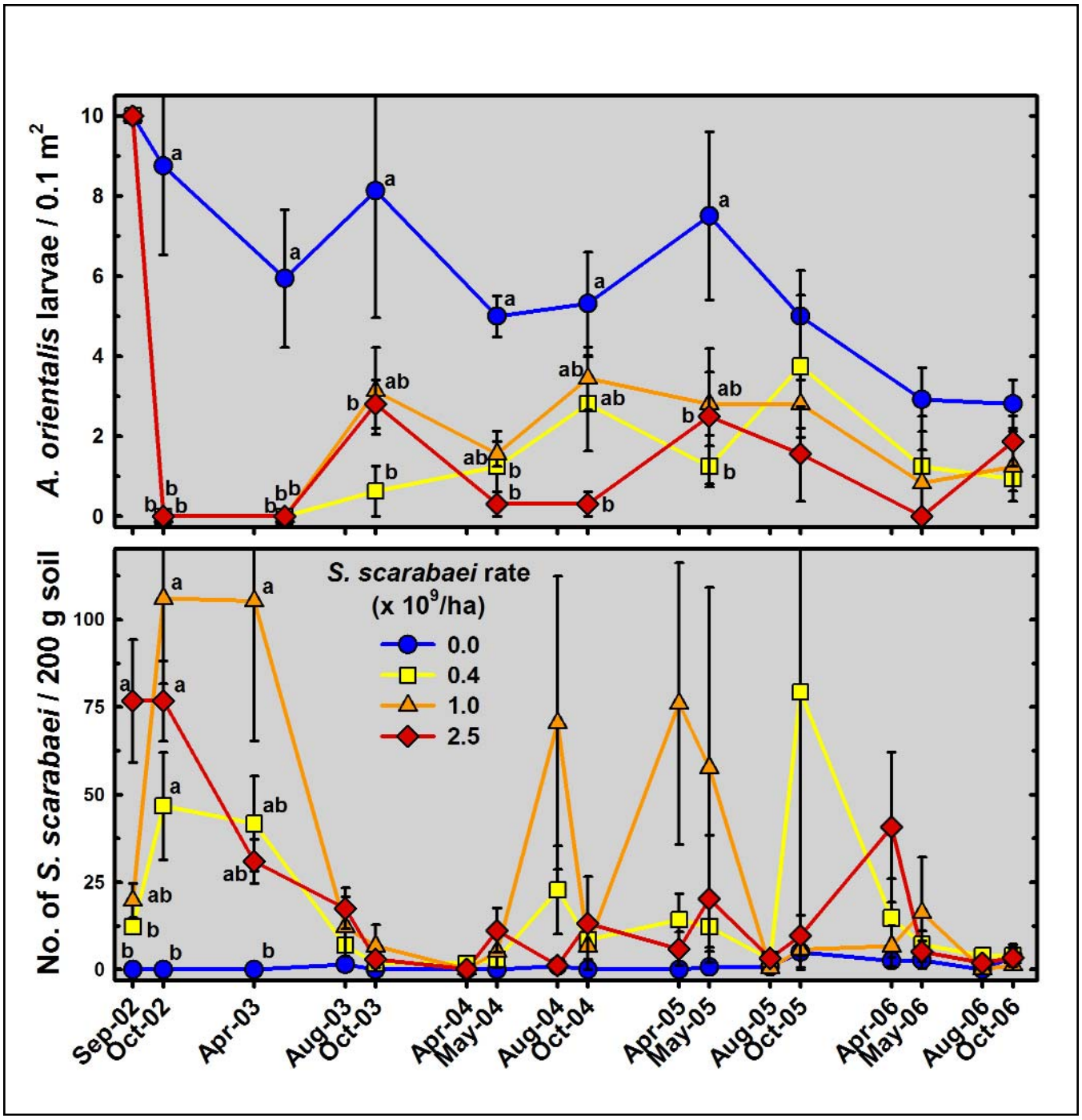


Figure 1. Population densities (± SE) of larval *Anomala orientalis* and the entomopathogenic nematode *Steinernema scarabaei* after application (mid-September 2002) of different *S. scarabaei* rates to turfgrass microplots seeded with late second- and early third-instar *A. orientalis* (10 per 0.1 m^2).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

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 m2, 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.

Long-term Effects on *A. orientalis* and *S. scarabaei* Populations

Experiment 1

A. orientalis recovery was significantly affected by *S. scarabaei* application rate within each sampling date until 978 days after treatment (DAT) (Figure 1). The highest *S. scarabaei* rate caused significant reductions in *A. orientalis* numbers at 31, 211, 610, and 760 DAT (94-100%), but not on the remaining dates (33-100%); the middle *S. scarabaei* rate caused significant reductions at 31 and 211 DAT (100%) but not on the remaining dates (35-71%); and the lowest *S. scarabaei* rates caused significant reductions at 31, 211, 399, and 978 DAT (83-100%) but not on the remaining dates (25-75%). During the fourth year, *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.

S. scarabaei numbers extracted from soil samples at 0 DAT 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) (Figure 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). 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 and had further declined by 399 DAT and 567 DAT. From 610 DAT until the end of the experiment, *S. scarabaei* numbers showed great fluctuation and variability within treatments. *S. scarabaei* was detected in every treated plot until 323 DAT. Thereafter, detection rates across all treated plots varied from 33% to 92%.

S. scarabaei was for the first time detected in the control plots at 323 DAT, then again at 695 DAT, and was recovered on most sampling dates starting at 978 DAT until the end of the experiment. However, recovery was generally very low and erratic.

Experiment 2

A. orientalis recovery was significantly affected by application rate mostly on the spring sampling dates (Figure 2). The highest *S. scarabaei* rate caused significant reductions in *A. orientalis* numbers at 34, 244, 612, 978, and 1492

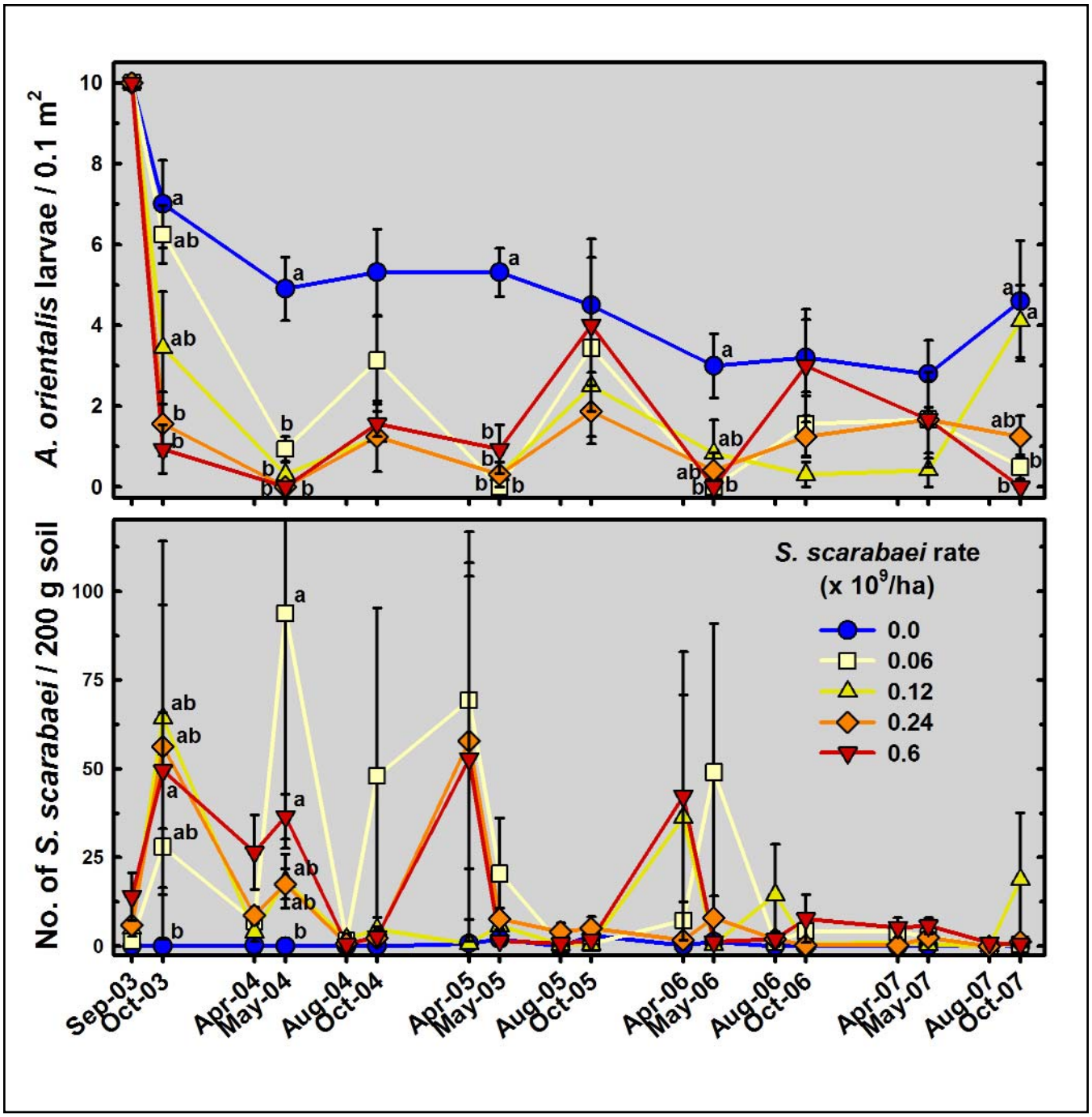


Figure 2. Population densities (± SE) of larval *Anomala orientalis* and the entomopathogenic nematode *Steinernema scarabaei* after application (mid-September 2003) of different *S. scarabaei* rates to turfgrass microplots seeded with late second- and early third-instar A. orientalis (10 per 0.1 m2). Means with the same letter within sampling date are not significantly different; no letters shown for dates without significant differences (P < 0.05, Tukey).

DAT (82-100%) but not on the remaining dates (5-71%); the second highest rate caused significant reductions at 34, 244, and 612 DAT (77-100%), but not on the remaining dates (33-77%); the third highest rate caused significant reductions at 244 and 612 DAT (93-94%), but not on the remaining dates (5-89%); and the lowest rate caused significant reductions at 244, 612, 978, and 1492 DAT (80-100%), 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. On none of the sampling dates did *A. orientalis* recovery differ among the different *S. scarabaei* rates.

S. scarabaei numbers extracted from soil samples at 0 DAT did not differ significantly between the control and the treatments. At 34 DAT, significantly more *S. scarabaei* were extracted from the highest *S. scarabaei* rate than from control plots. 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. At 244 DAT, *S. scarabaei* numbers had increased in all *S. scarabaei* treatments compared to 201 DAT and were significantly higher than in the control in the highest and the lowest *S. scarabaei* treatments.

By 329 DAT, *S. scarabaei* numbers had dropped dramatically in all *S. scarabaei* treatments. From 329 DAT until the end of the experiment no significant differences among treatments were detected. During this period *S. scarabaei* numbers were generally low but there were occasional spikes. *S. scarabaei* was recovered from 88-100% of the treated plots until 244 DAT, but thereafter recovery rates were generally lower with 6-75%. *S. 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 and was no longer detected thereafter.

Experiment 3

For all data combined, all *S. scarabaei* treatments had significantly lower *A. orientalis* recovery than the control without significant differences among rates (Figure 3). When comparing treatment effects within each sampling date, the highest *S. scarabaei* rate caused significant reductions in *A. orientalis* numbers at 31 and 249 DAT (93-96%), 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, 249, 398, and 616 DAT (80-100%), 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 and *A. orientalis* recovery did not differ among treatments.

S. scarabaei numbers extracted from soil samples 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 were 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. Until 249 DAT, *S. scarabaei* was recovered from 80% to 93% of the treated plots. Thereafter, recovery varied between 0 and 47%. *S. scarabaei* was recovered from the control plots at 0, 201, 328, and 1130 DAT; however, each time only one nematode was detected.

Other Scarab Species, Other Nematode Species, and Nematode Infections

In all three experiments, numbers of other scarab species recovered (*P. japonica* and northern masked chafer, *C. borealis*) were too low and too erratic to be included in the analysis and presentation. Baiting also revealed the presence of endemic populations of *H. bacteriophora* and *S. carpocapsae* in all experiments. *H. bacteriophora* was usually recovered from the majority of plots and often in higher numbers than *S. scarabaei* in the treated plots. *S. carpocapsae* recovery was more variable and tended to be lower.

Densities of both species significantly varied with sampling date but were not affected by *S. scarabaei* application rate. During the first evaluation (31-34 DAT), especially in the first experiment, numerous recovered *A. orientalis* were *S. scarabaei*-infected. However, on the following sampling dates, recovery of infected larvae was much lower and more variable. In all experiments combined, only two *H. bacteriophora*-infected larvae was recovered.

Conclusions

The scarab-adapted entomopathogenic nematode *S. scarabaei* provided 77-100% control of *A. orientalis* larvae within 31-34 days of appli-

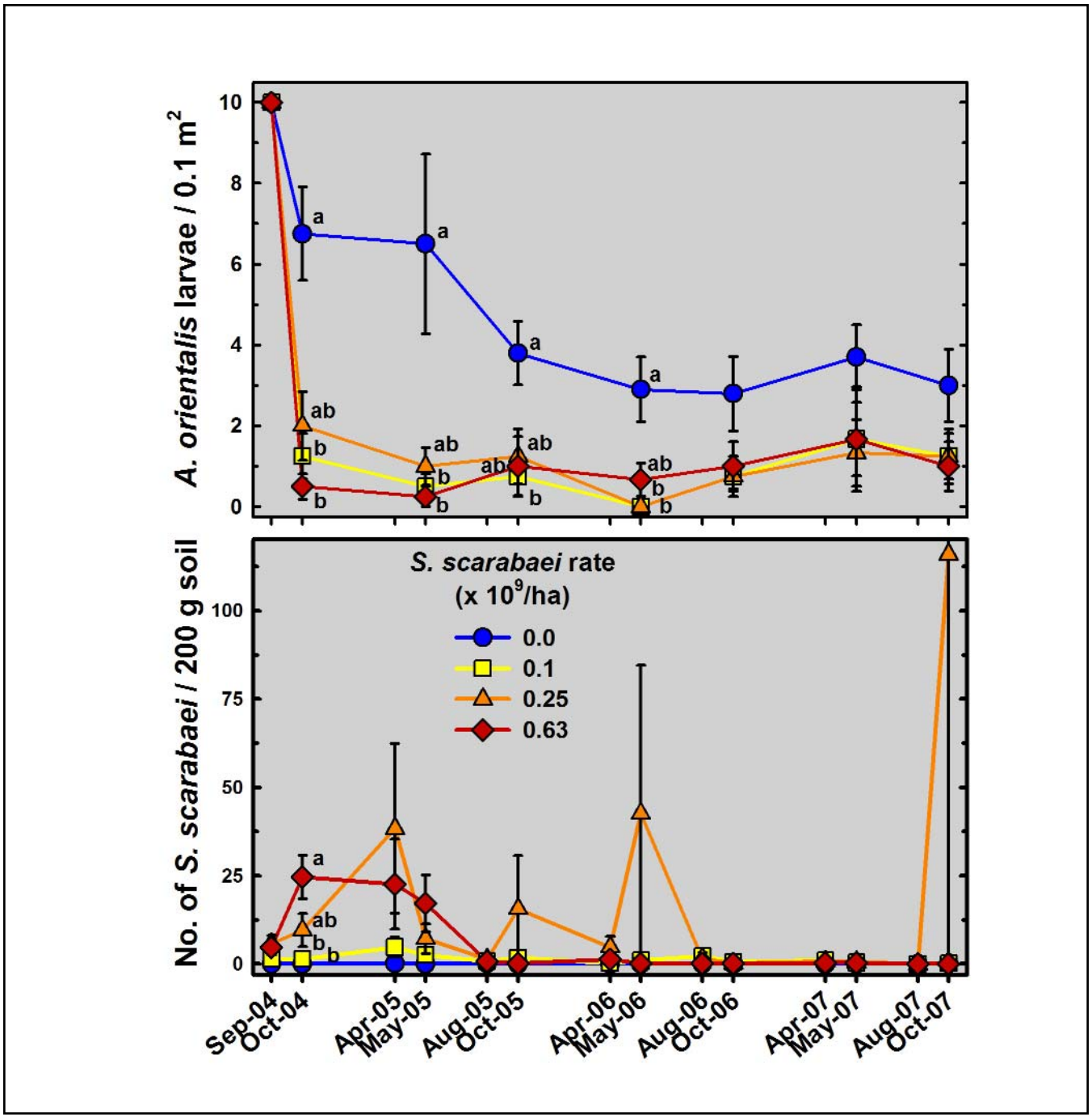


Figure 3. Population densities (± SE) of larval *Anomala orientalis* and the entomopathogenic nematode *Steinernema scarabaei* after application (mid-September 2004) of different *S. scarabaei* rates to turfgrass microplots seeded with late second- and early third-instar *A. orientalis* (10 per 0.1 m^2).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

cation at very low application rates (0.25 to 2.5 × 10^9 IJs/ha). Even more consistent control was observed in the following spring at even lower rates (0.1 to 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. More than 2 years after application, significant control was only observed occasionally, but larval densities remained numerically lower in all treatments than in the untreated plots on all sampling dates. *S. scarabaei* numbers recovered from soil samples during the first year after application were fairly

consistent, showing 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 became more variable over time, presumably due to increasing patchiness.

Compared to previous studies (14, 16), the short-term effects of *S. scarabaei* on *A. orientalis* were stronger in this study. These increases in efficacy should be related to the 10-13 day longer period between application and evaluation, giving the nematodes enough time to recycle with emerging progeny causing additional white grub mortality. Indeed, *S. scarabaei* densities increased between 0 DAT and the first evaluation date and numerous *S. scarabaei*-infected larvae were recovered at the first evaluation. Recovery of infected larvae on later sampling dates was lower since infections were probably less synchronized.

The effect of *S. scarabaei* on *A. orientalis* populations may have been limited by two factors. First, in all experiments, *A. orientalis* populations were never higher than 10 larvae per 0.1 m^2 in the untreated plots, most of the time closer to 5 larvae per 0.1 m^2. It is possible that higher larval populations may have increased *S. scarabaei* recycling. That, in turn, would have improved long-term control as the nematode should have a positive feedback 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.

More effective long-term suppression of white grub populations by *S. scarabaei* is likely to be limited by its poor survival during the summer months which should limit the nematode's impact on the new grub 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* poorly infects and poorly reproduces in other typical turfgrass insects such as billbug larvae, sod webworms, and cutworms (15). *S. scarabaei* also does not infect *A. orientalis* (and *P. japonica*) larvae that have purged their intestine in preparation for pupation, prepupae, and pupae (17) 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* (17), but progeny production from second-instars is only about 20% of that from third instars (Koppenhöfer, unpublished data). Thus, IJs produced from infections during fall or spring have to survive into mid-August before significant reproduction may occur. Nevertheless, enough IJs 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 *H. bacteriophora* and *S. carpocapsae* populations were regularly recovered in all experiments, often in higher numbers than *S. scarabaei*. However, the lack of correlation between their numbers and *A. orientalis* numbers indicated that they had no significant impact on *A. orientalis* populations. *H. bacteriophora* has shown limited effects on *A. orientalis* after inundative releases whereas *S. carpocapsae* is ineffective against white grubs in general (8).

Because of 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 experiments combined only two recovered scarab larvae were infected by *H. bacteriophora* and none by *S. carpocapsae*. But the three nematode species were able to coexist for the duration of our experiments. *S. carpocapsae* and *H. bacteriophora* probably persisted primarily on other turfgrass insects such as larvae of billbugs, sod webworms, and cutworms.

Overall, our study further underscores *S. scarabaei*'s great potential as a white grub biocontrol agent, 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 *S. scarabaei* can be mass produced economically, it could be released periodically (e.g., every 2-4 years) in areas with recurrent grub infestations to provide long-term suppression that should be safer and, due to the low release rates required, more economical than the use of synthetic insecticides.

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