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Entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) offer a non-toxic, environmentally safe, and IPM-compatible alternative to synthetic insecticides in turfgrass. Rutgers University scientists investigate the effects of soil moisture and soil type on the infectivity and persistence of nematodes for short- and long-term suppression of white grubs. Shown above is a white grub infected with *Steinernema scarabaei.*

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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 290 projects at a cost of $25 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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Nematodes for White Grub Control: Effects of Soil Type and Soil Moisture on Infectivity and Persistence

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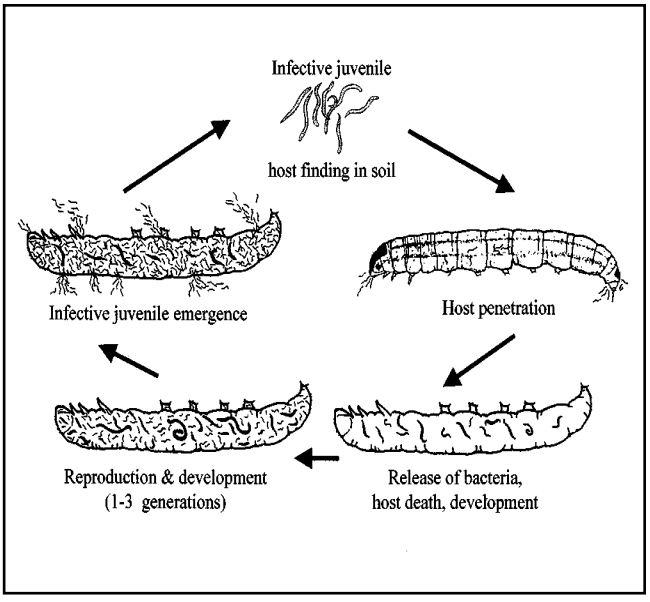
SUMMARY

Rutgers University scientists investigate the effects of soil moisture and soil type on the infectivity and persistence of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) for short- and long-term suppression of white grubs. Their findings include:

- The entomopathogenic nematode *Steinernema scarabaei* showed high virulence across a wide range of substrate types in laboratory and greenhouse studies. While *S. scarabaei* infectivity tended to decline from the coarser sandy soils to the finer clay soils, high mortality was observed in greenhouse pot experiments even in the finest soils. Only in highly acidic sand (pH 3.9) and highly organic potting mix did *S. scarabaei* infectivity decline significantly, however, still caused significant mortality.
- *Heterorhabditis bacteriophora* showed similar infectivity from the coarser to the finer soils, was also negatively affected in acidic sand, but was most infective in a highly organic potting mix.
- Both *S. scarabaei* and *H. bacteriophora* were most infective at moderate soil moisture levels and less in saturated soil and drier soil. However, the infectivity range of *S. scarabaei* extended further into the dry range with significant activity even in dry soil (-3,000 kPa).
- *S. scarabaei* also showed high persistence levels in all substrate types and soil moisture levels, whereas *H. bacteriophora* generally was short with good persistence only in the drier soils.
- *S. scarabaei* has excellent potential for short-term and long-term suppression of white grub populations.

A complex of white grub species are the most widespread and destructive turfgrass insect pests in the United States. As key species have been considered the Japanese beetle, *Popillia japonica*, throughout much of the eastern US, and masked chafers, *Cyclocephala spp.*, in the Midwest and western states (11, 12). However, in the Northeast and along the eastern seaboard, oriental beetle, *Anomala orientalis*, European chafer, *Rhizotrogus majalis*, and Asiatic garden beetle, *Maladera castanea*, have become similarly important pests. At present, synthetic insecticides are still the primary means of controlling white grubs. But due to the implementation of the Food Quality Protection Act of 1996 (FQPA), golf turf managers have already lost many and may lose more options for curative white grub control.

Neonicotinoid insecticides (imidacloprid, clothianidin) and insect growth regulators (halofenozide) are less hazardous than organophosphates and carbamates, but they are only effective when used preventively, resulting in the treatment of large areas that otherwise would have needed only partial or no treatment. Preventive applications of these compounds are expensive, increase the chances of resistance development, and may have unintended environmental consequences.



Entomopathogenic nematodes used in this study infect the insect larvae and proliferate as bacteria kills the host insect. Newly released nemotodes are then released into the soil as the insect cadaver deteriorates allowing infective juveniles to find another susceptible host.

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In the long-term, these insecticides' 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, oriental beetle and European chafer cannot be effectively controlled with halofenozide and the Asiatic garden beetle is resistant to halofenozide and imidacloprid (1, 4).

Entomopathogenic nematodes as an alternative to chemical white grub control

Entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) offer a non-toxic, environmentally safe, and IPM-compatible alternative to synthetic insecticides in turfgrass. These nematodes occur in natural and agricultural soils around the world and are used for biological control of insects, primarily soil-dwelling insects and in an inundative approach (3).

The only free-living stage of entomopathogenic nematodes is the infective juvenile whose purpose is to persist in the soil until it can locate and infect a suitable host. After penetrating into the host's body cavity the infective juvenile regurgitates species-specific symbiotically associated bacteria, and nematode and bacteria cooperate to kill the host within a few days. The developing nematodes feed on the bacteria and host tissues digested by the bacteria and develop through up to three generations, until hundreds to hundred of thousands of new infective juveniles emerge from the depleted host cadaver to search for new hosts.

Research in the US has shown that these nematodes can be as effective as synthetic insecticides against Japanese beetle larvae (2). However, recent research has shown that masked chafers, oriental beetle, European chafer, Asiatic garden beetle, and other white grub species are less susceptible than the Japanese beetle to the commonly used nematode species such as *Heterorhabditis bacteriophora* and *Steinernema glaseri* (4, 5, 7). The mixed results with nematodes against white grubs observed in the past are certainly in part due to their use against species other than the Japanese beetle.

***Steinernema scarabaei*: a new nematode highly virulent to white grubs**

We have recently isolated a new nematode species, *Steinernema scarabaei*, from epizootics in Japanese and oriental beetle larvae in New Jersey turfgrass areas. Laboratory studies indicated that *S. scarabaei* is specialized to scarab larvae as hosts. *S. scarabaei* was highly virulent to and reproduced very well in oriental beetle and

Substrate type	Percent Sand/Silt/Clay	OM	pH	% moisture (w/w) at -10 kPa	% moisture (w/w) at saturation
Loamy Sand	84 / 11 / 5	1.1	6.3	9	19
Sandy Loam[1]	61 / 27 / 12	2.3	5.9	15	20
Loam	38 / 44 / 18	2.5	6.7	23	33
Silt Loam	21 / 60 / 19	2.1	7.0	22	34
Clay Loam	27 / 37 / 36	3.7	7.0	27	42
Potting Mix[2]	--- / --- / ----	67.5	5.3	57	120
Acidic Sand	93 / 7 / 0	5.1	3.9	11	20

[1]Percent moisture (by weight) at -1, -100, -1,000, -3,000 kPa: 20, 10, 7, 4.2

[2]Pine bark : Peat moss : Sand (grit) = 3 : 2 : 1 (by volume)

Table 1. Characteristics of substrate types used

Japanese beetle larvae, but its virulence to and reproduction in the larvae or adults of species from various other families of Coleoptera, Lepidoptera, and other insect orders was generally low to non-existent. *S. scarabaei* is well adapted to infecting sedentary hosts below the soil surface but poorly performs against mobile hosts on the soil surface. *S. scarabaei* caused significant mortality to and reproduced in oriental beetle larvae at 59 to 82°F (optimum 64 to 77°F) (6).

In laboratory, greenhouse, and field studies, *S. scarabaei* has shown exceptional virulence to a wide range of white grub species including Japanese beetle, oriental beetle, European chafer, Asiatic garden beetle, and several May/June beetle species. It dramatically outperformed any other nematode species tested in greenhouse and field studies even at rates as low as one fourth of the other species applied (4, 5, 7, 8). In ongoing field studies supported by USGA we have seen significant suppression of oriental beetle larval populations by *S. scarabaei* at least one year, often two years, after application. This long term effect is due to the high virulence of *S. scarabaei*, the long persistence of its infective juveniles, and its effective reproduction and recycling in the infected white grubs.

Factors affecting nematode efficacy and survival in the field

The performance of entomopathogenic nematode can be affected by many environmental factors. Two of the most important factors are soil moisture and soil type/texture. In soil, infective juveniles move through the water film that coats the interstitial spaces. If this film becomes too thin (in dry soil) or the interstitial spaces are completely filled with water (in water-saturated soil), nematode movement can be restricted. In field studies, soil moisture is positively related to *H. bacteriophora* efficacy against Japanese beetle larvae (2). Infective juveniles can survive desiccation to relatively low moisture levels if water removal is gradual giving them time to adapt to an inactive stage.

Generally, nematode survival and dispersal tend to be lower in fine-textured soils. But the effect of soil moisture and texture on nematode infectivity and survival varies with nematode species (9, 10) and may depend on nematode size, behavior, and physiology. However, other factors can also play a role. In turfgrass trials against Japanese beetle larvae, *H. bacteriophora* was more effective in fine-textured soils than sandy soil probably because finer soils retain moisture better and restrict nematode movement to the upper soil layers where most of the white grubs are found (2).

Studies on the effect of soil type and soil moisture on nematode performance

To improve the predictability of *S. scarabaei* applications in the field, both for short-term and long-term white grub management, we conducted a series of laboratory and greenhouse experiments studying the effect of different soil types and moisture levels on the infectivity and survival of this species. For comparison, the well known and widely available species *H. bacteriophora* was included in the study.

Five typical mineral soils were collected from turfgrass areas, acidic sand from a blueberry field, and a typical potting mix from a nursery. The substrates were air-dried, sieved, sterilized, and aerated before use. Soil characteristics and soil moisture release curves were established (Table 1). Third-instar oriental beetles and Japanese beetles were collected in turf areas and stored individually at 10°C for one to ten weeks in a mixture of organic compost and loamy sand. The entomopathogenic nematodes *H. bacteriophora* (GPS11 strain) and *S. scarabaei* (AMK001 strain) were cultured and stored following standard procedures. All experiments, unless otherwise mentioned, were conducted in the laboratory at room temperature (20 - 24°C).

Effect of soil type on nematode infectivity

Two laboratory experiments tested the effect of six substrates prepared at -10 kPa (approximate field capacity) on nematode infec-

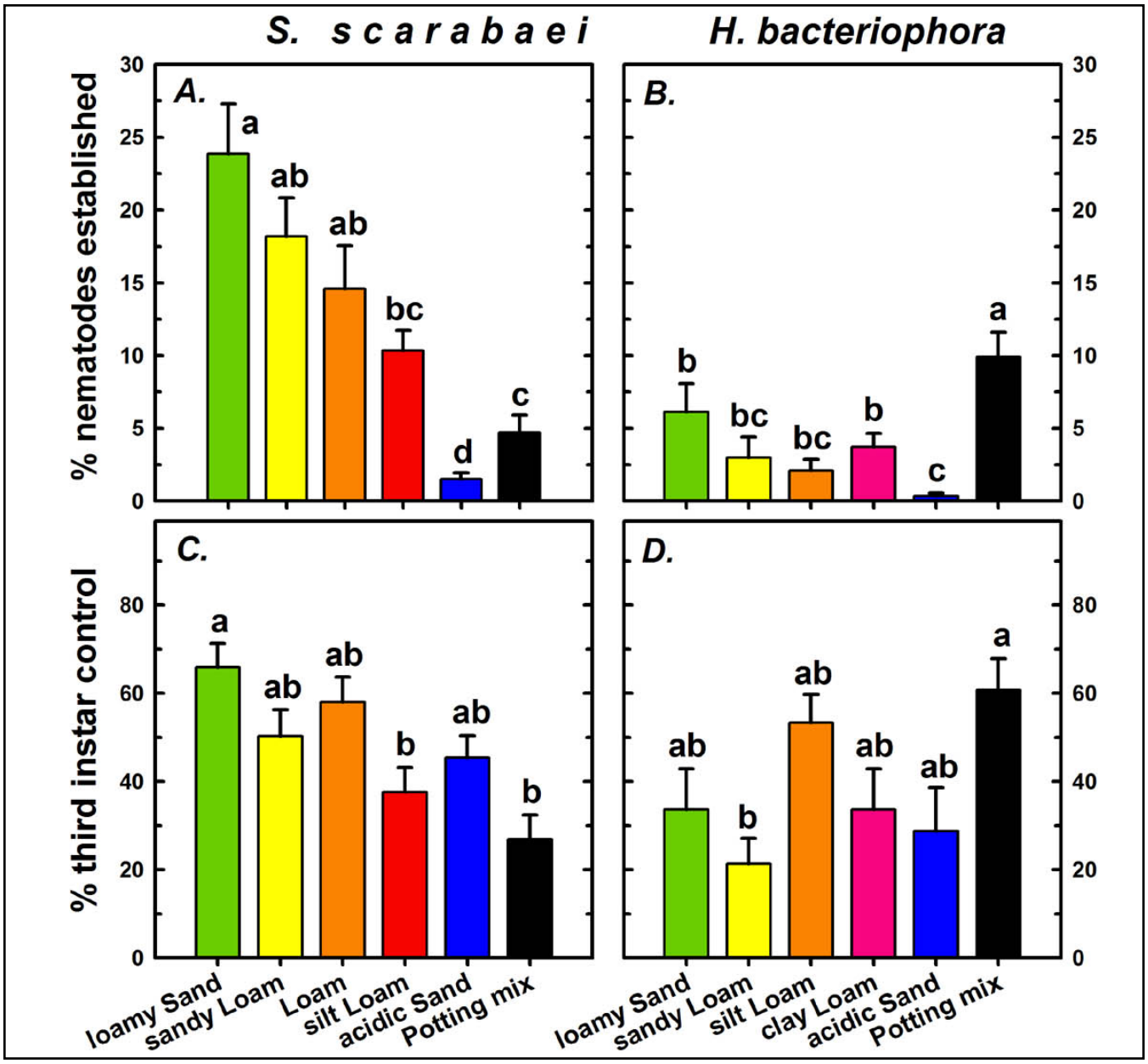

Figure 1. Effect of different substrates on infectivity (A-B) and efficacy (C-D) of the entomopathogenic nematode *Steinernema scarabaei* against third-instar oriental beetle (A,C) and *Heterorhabditis bacteriophora* against third-instar Japanese beetle (B, D). Columns with same letter are not significantly different ($P < 0.05$).

tivity. The substrates were filled into plastic vials (43-mm diameter) and uniformly compacted to a depth of 63 mm. Into each vial, one third-instar white grub was released, and one day later nematodes in 100 µl tap water were pipetted into the soil. Experiment 1 tested the infectivity of *S. scarabaei* (0 or 200 infective juveniles per vial) against oriental beetle larvae. Experiment 2 tested the infectivity of *H. bacteriophora* (0 or 500 infective juveniles per vial) against Japanese beetle larvae. At seven days after treatment (DAT), the larvae were recovered and infected larvae were dissected and digested in a pepsin solution, and the number of nematodes established in them counted. Each experiment had 15 vials per treatment and was conducted twice.

The number of *S. scarabaei* established in the larvae was significantly lower in acidic sand than in potting mix, and significantly lower in potting mix than in loam, sandy loam, and loamy sand, and significantly lower in silt loam than in loamy sand (Figure 1A). Larval mortality fol-

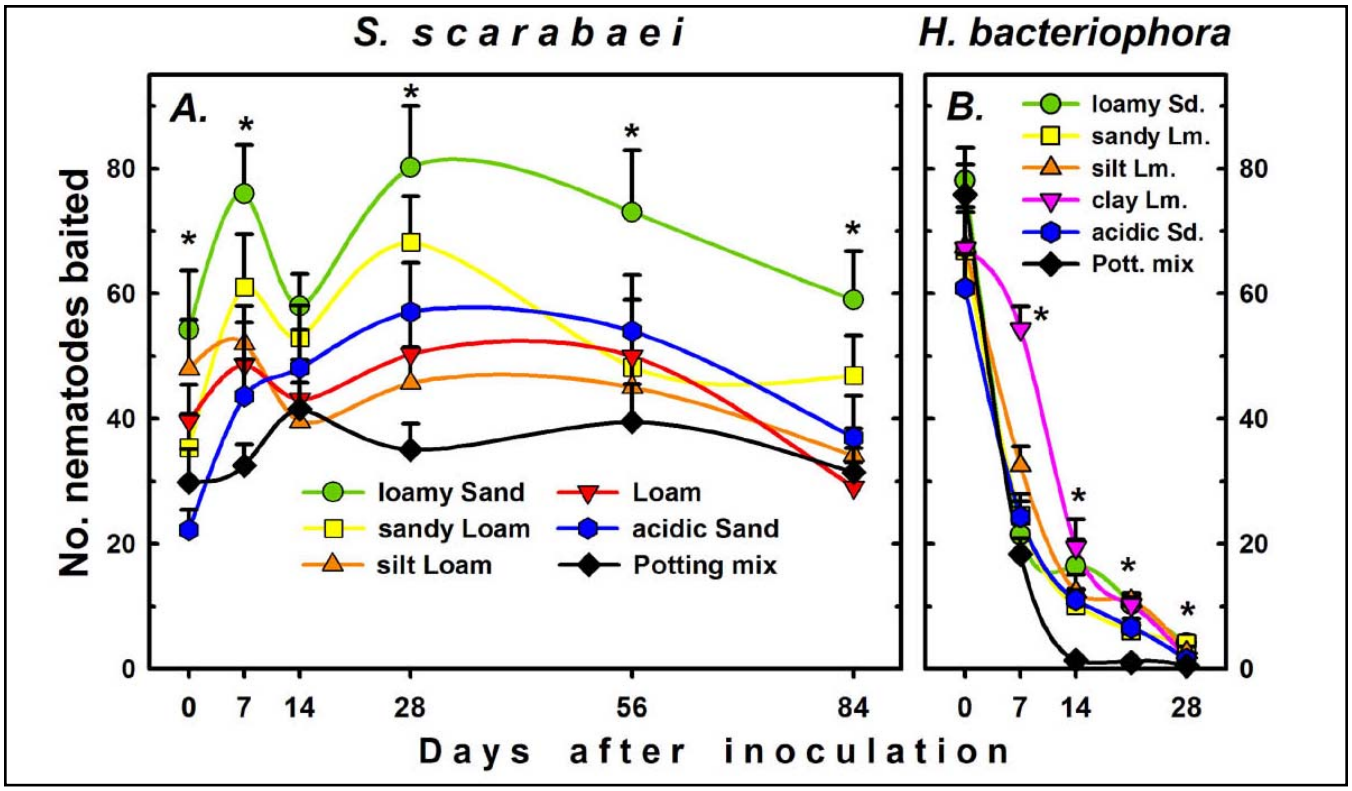

Figure 2. Persistence of *Steinernema scarabaei* (A) and *Heterorhabditis bacteriophora* (B) in different substrates. * indicates significant differences in recovery among substrates types per baiting date ($P < 0.05$;). A significant decline in recovery was found in no substrate for *S. scarabaei* and in all substrates for *H. bacteriophora*.

lowed a very similar pattern with significantly lower mortality in acidic sand (50%) and potting mix (67%) than in the other soils (90 - 100%). *H. bacteriophora* establishment in larvae (Figure 1B) was the highest in potting mix and the lowest in acidic sand and did not differ significantly among loamy sand, sandy loam, silt loam, and clay loam. Larval mortality also was the highest in potting mix (93%) and the lowest in acidic sand (13%) and did not differ significantly among loamy sand, sandy loam, silt loam, and clay loam (57 - 63%).

Two greenhouse experiments (28°C/18°C day/night; 14/10 h light/dark) were conducted in 1-liter pots (100 cm^2 surface area) with perennial ryegrass growing on the various substrates. The pots were watered every two to three days to saturation to maintain a similar soil moisture (i.e., approximate field capacity) in all the substrate types. Five third-instar larvae were released per pot three days before treatment. Experiment 1 tested *S. scarabaei* (0 or 156 infective juveniles per pot) against oriental beetle. Experiment 2 tested *H. bacteriophora* (0 or 625 infective juveniles per pot) against Japanese beetle. The pots were destructively sampled at 14 DAT to determine the number of surviving larvae. Both experiments were conducted twice with 10 - 12 replicates per treatment.

Mortality in the greenhouse experiment followed a similar trend as mortality in the laboratory experiment except that the negative effect of acidic sand was less pronounced, possibly modulated by the presence of grass roots (Figure 1). *S. scarabaei* caused higher mortality in loamy sand than in silt loam and potting mix with sandy loam, loam, and acidic sand not significantly different from either group (Figure 1C). *H. bacteriophora* caused higher mortality in potting mix than in sandy loam and the remaining soils were not significantly different from either group (Figure1D).

Effect of soil type on nematode persistence

Two laboratory experiments tested the effect of six soil types (at -10 kPa) on persistence of *S. scarabaei* and *H. bacteriophora*. One hun-

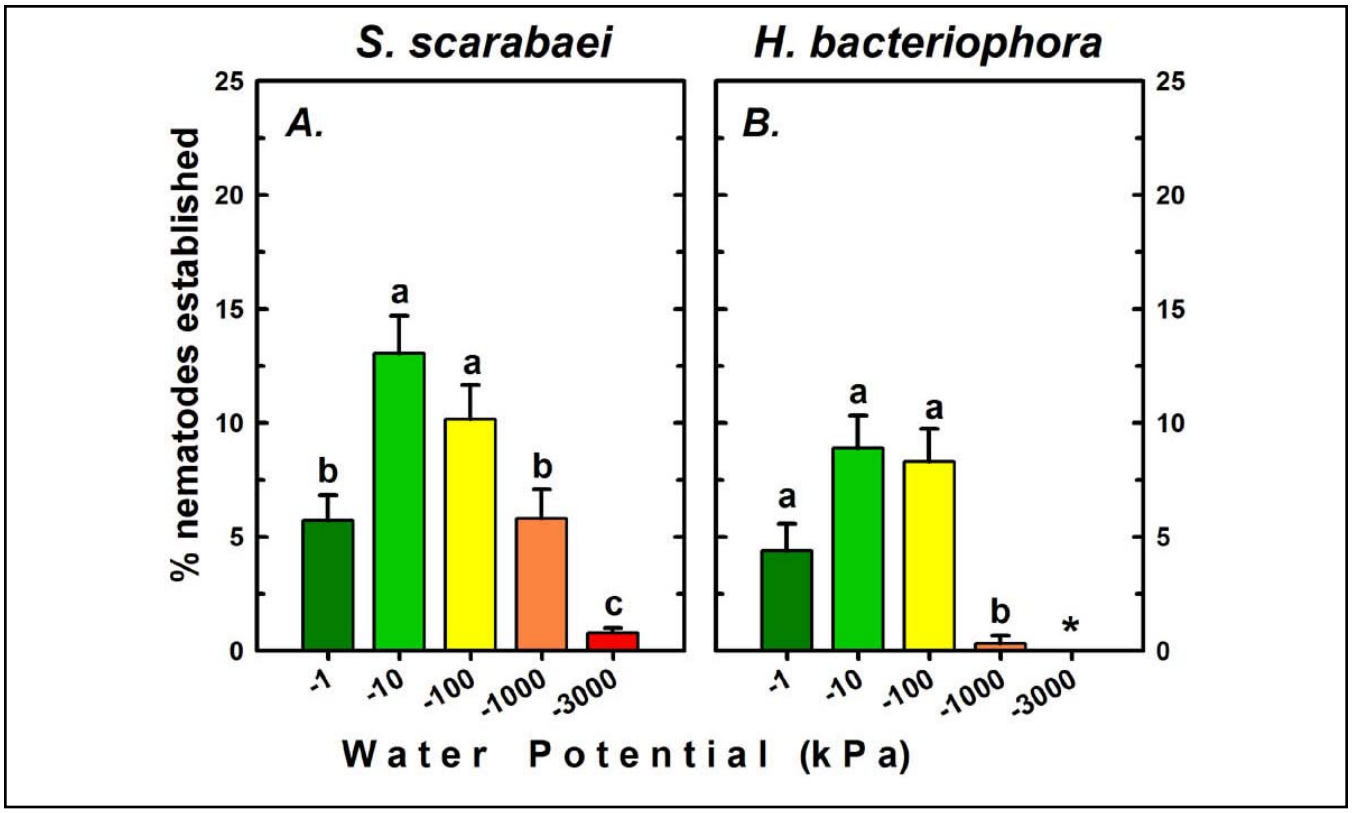

Figure 3. Effect of soil water potential on infectivity of the entomopathogenic nematodes *Steinernema scarabaei* (A) and *Heterorhabditis bacteriophora* (B) in different substrates against third-instar *Popillia japonica*. Columns with same letter are not significantly different ($P < 0.05$). * indicates that no infection and mortality occurred at these data points.

dred g of substrate were filled into 200-ml plastic cups and uniformly compacted. Cups with potting mix received only 33 g due to the lower density of this substrate. After adding 200 infective juveniles per cup, the cups were kept in plastic boxes with wet paper towel. At different times after treatment (Figure 2), five cups from each treatment were opened and the soil baited with five wax moth larvae for four 3-day baiting rounds. Infected larvae were dissected and digested to count the number of nematodes established in them.

S. scarabaei showed excellent persistence with no significant decline in recovery over time in any of the substrates (Figure 2A). Over the entire experiment, recovery was significantly higher in loamy sand than all other soils, and significantly higher in sandy loam than in potting mix. Recovery in the remaining substrates did not differ significantly from either sandy loam or potting mix. *H. bacteriophora* persistence was much shorter, and recovery significantly declined in all substrates (Figure 2B). Recovery declined the quickest in potting mix and the slowest in clay loam. Overall, recovery was significantly higher in clay loam than in sandy loam and acidic sand and was lower in potting mix than in all other soils.

Effect of soil moisture on nematode infectivity

Sandy loam was prepared at different soil water potentials (from wettest to driest: -1, -10, -100, -1,000, -3,000 kPa), allowed to equilibrate for four days, mixed again, filled into plastic vials (43-mm diameter), and uniformly compacted to a depth of 63 mm. Into each vial, one third-instar Japanese beetle was released and, one day later, 0 nematodes, 200 *S. scarabaei* or 1,000 *H. bacteriophora* were pipetted into the soil. At seven days after treatment (DAT), the larvae were recovered and infected larvae were dissected and digested in a pepsin solution, and the nematodes established in them counted. Each experiment had 15 vials per treatment and was conducted twice.

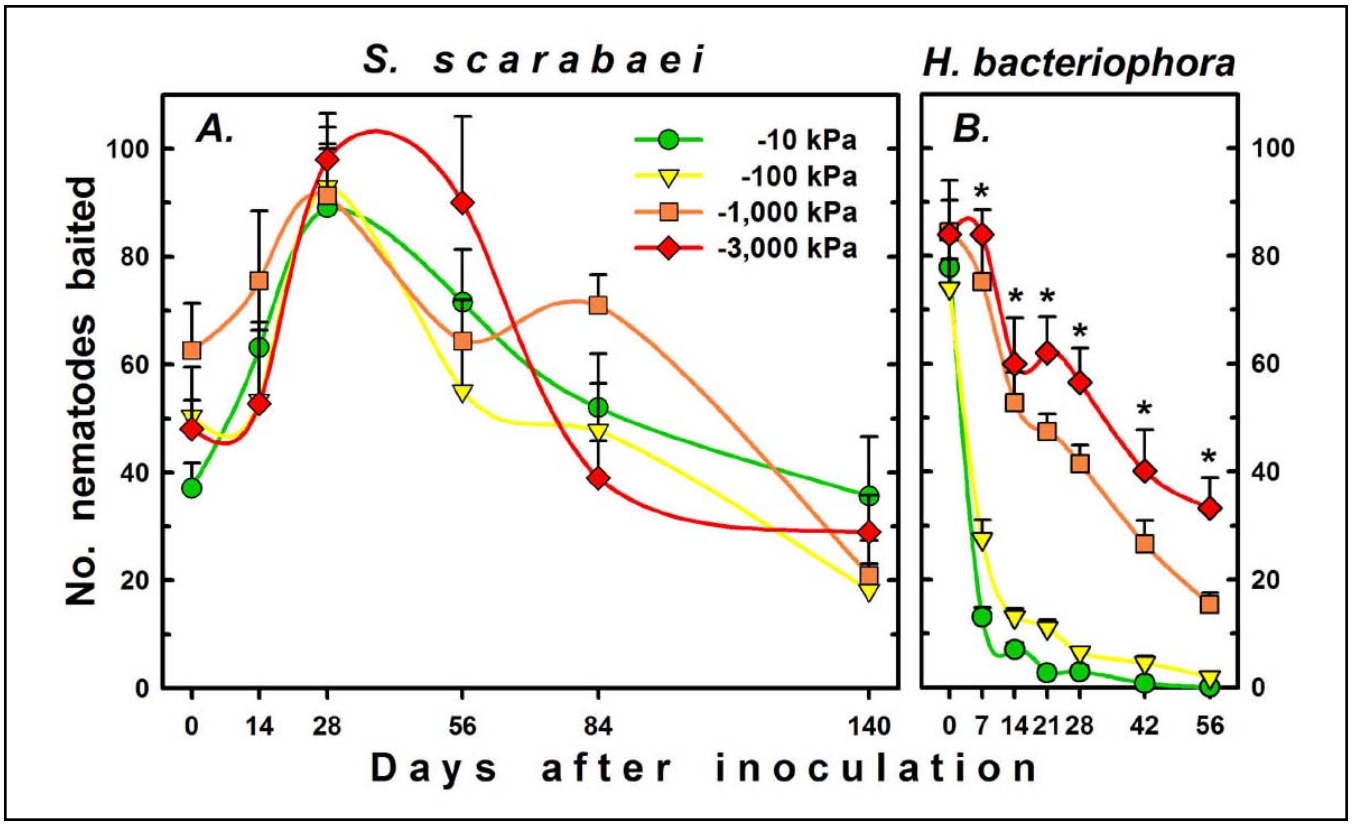

Figure 4. Persistence of *Steinernema scarabaei* or *Heterorhabditis bacteriophora* in sandy loam at four soil water potentials. * indicates significant differences in recovery among water potential per baiting date ($P < 0.05$). A significant linear decline in recovery was found for -100 kPa and -1,000 kPa in *S. scarabaei* and for -10 kPa to -3,000 kPa in *H. bacteriophora* ($P < 0.05$).

The number of *S. scarabaei* established in larvae was higher at -10 kPa and -100 kPa than at -1 kPa and -1000 kPa but was the lowest at -3,000 kPa (Figure 3A). Larval mortality by *S. scarabaei* was significantly higher at -100 kPa (100%), -10 kPa (97%), and -1,000 kPa (87%), than at -3,000 kPa (50%), with mortality at -1 kPa (77%) not significantly different from either group. *H. bacteriophora* establishment followed a similar pattern as for *S. scarabaei* at -1 kPa to -100 kPa, but was more restricted in drier soil with very low establishment at -1,000 kPa and no establishment at -3,000 kPa (Figure 3B). Similarly, larval mortality by *H. bacteriophora* was significantly higher at -1 kPa (57%), -10 kPa (77%), and -100 kPa (67%) than at -1,000 kPa (17%) with no mortality at -3,000 kPa.

Effect of soil moisture on nematode persistence

Sandy loam was prepared at different soil water potentials (from wettest to driest: -10, -100, -1,000, -3,000 kPa), allowed to equilibrate for four days, mixed again, and 100 g of soil filled into 200-ml plastic cups and uniformly compacted. After adding 200 infective juveniless of *S. scarabaei* or *H. bacteriophora* per cup, the cups were stored and baited at different intervals (Figure 4) as described for the soil type persistence experiments. There were five cups per treatment and sampling date in each of two trials.

S. scarabaei persistence was excellent and was not affected by soil water potential (Figure 4A). Averaged across all soil water potentials, *S. scarabaei* recovery initially increased but declined thereafter and was significantly lower on day 140 than all other days. *H. bacteriophora* significantly declined much quicker at all soil water potentials (Figure 4B) but declined the fastest at -10 kPa, somewhat slower at -100 kPa, and much slower at -1,000 kPa and particularly -3,000 kPa.

Conclusions

Our observations further illuminate the excellent potential of *S. scarabaei* for short-term and long-term suppression of white grub populations. In addition to its high virulence against a wide range of white grub species, it also showed high virulence across a wide range of substrate types. While *S. scarabaei* infectivity tended to decline from the coarser sandy soils to the finer clay soils, significant mortality was observed in greenhouse pot experiments even in the finest soils. Even in clay loam, *S. scarabaei* was only somewhat less infective in laboratory and greenhouse experiments (9, 10). Only in highly acidic sand (pH 3.9) and highly organic potting mix did *S. scarabaei* infectivity decline significantly, however, still caused significant mortality.

In comparison, *H. bacteriophora* (at considerably higher rates and against the more susceptible Japanese beetle) showed similar infectivity from the coarser to the finer soils, was also

Nematode-infected white grubs become flaccid and turn a characteristic color (yellowish for *S. scarabaei*, above; reddish-brown for *H. bacteriophora*).

negatively affected in acidic sand, but was the most infective in the potting mix. Both *S. scarabaei* and *H. bacteriophora* were most infective at moderate soil moisture and less in saturated soil and drier soil. However, the infectivity range of *S. scarabaei* extended further into the dry range with significant mortality even in dry soil (-3,000 kPa) where *H. bacteriophora* did not cause infections. *S. scarabaei* also showed excellent persistence over all substrate types and soil moisture levels, whereas *H. bacteriophora* generally persisted much shorter with more useful persistence levels only in the drier soils where it becomes inactive.

As indicated earlier, *S. scarabaei* has already shown good potential for long-term white grub suppression in turfgrass field studies (study supported by USGA) with significant suppression of oriental beetle larval populations at least one year, often two years, after application. The present study indicates that this long-term effect should be achievable over a wide range of soil conditions. The major problem still to overcome in the commercialization of *S. scarabaei* is the development of effective mass production technology which has proven to be difficult and may require more in-depth studies on *S. scarabaei*'s nutritional requirements.

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