Research *You Can Use*

Nematodes for White Grub Control

Rutgers University scientists investigate soil effects on nematode suppression of white grubs.

BY ALBRECHT M. KOPPENHÖFER AND EUGENE M. FUZY

At present, synthetic insecticides are still the primary means of controlling white grubs. With the implementation of the Food Quality Protection Act of 1996, golf turf managers have already lost many options for curative white grub control.

Species of white grubs are the most widespread and destructive turfgrass insect pests in the United States. Key species are the Japanese beetle (*Popillia japonica*) throughout much of the eastern United States, and masked chafers (*Cyclocephala spp.*) in the Midwest and western states. However, in the Northeast and along the eastern seaboard, the 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 options, and may lose more, 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. 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.

ENTOMOPATHOGENIC NEMATODES AS AN ALTERNATIVE

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.

The only free-living stage of entomopathogenic nematodes is the infective juvenile that must 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. The 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 hundreds of thousands of new infective juveniles emerge from the depleted host cadaver to search for new hosts.

Research in the U.S. has shown that these nematodes can be as effective as synthetic insecticides against Japanese beetle larvae. However, recent research has shown that the masked chafer, 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*.

STEINERNEMA SCARABAEI: A NEW HIGHLY VIRULENT NEMATODE

We have recently isolated a new nematode species, *Steinernema scarabaei*, living in Japanese and oriental beetle larvae in New Jersey turfgrass areas. This nematode was highly virulent to and reproduced very well in oriental beetle and 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. It is well adapted to infecting sedentary hosts below the soil surface, but performs poorly against mobile hosts on the soil surface.

In laboratory, greenhouse, and field studies, *S. scarabaei* has shown exceptional virulence to a wide range of white grub species, including the 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. In ongoing field studies supported by the 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.

Rutgers University scientists investigated 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*.

FACTORS AFFECTING NEMATODE EFFICACY AND SURVIVAL

The performance of entomopathogenic nematodes 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 pore spaces. If this film becomes too thin (in dry soil) or the pore spaces are completely filled with water (in water-saturated soil), nematode movement can be restricted. In field

studies, soil moisture was positively related to *H. bacteriophora* efficacy against Japanese beetle larvae. Infective juveniles can survive dessiccation 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 and may depend on nematode size, behavior, and physiology. 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.

STUDIES ON THE EFFECT OF SOIL TYPE AND SOIL MOISTURE

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. Soil characteristics and soil moisture release curves were established. Third-instar oriental beetles and Japanese beetles were collected in turf areas and stored individually at 50°F for one to ten weeks in a mixture of organic compost and loamy sand. The nematodes *H. bacteriophora* (GPS11 strain) and *S. scarabaei* (AMK001 strain) were cultured and stored following standard procedures.

EFFECT OF SOIL TYPE ON NEMATODE INFECTIVITY

Two laboratory experiments tested the effect of six substrates on nematode infectivity. 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 were counted.

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 followed 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 were with perennial ryegrass growing on the various substrates. Experiment 1

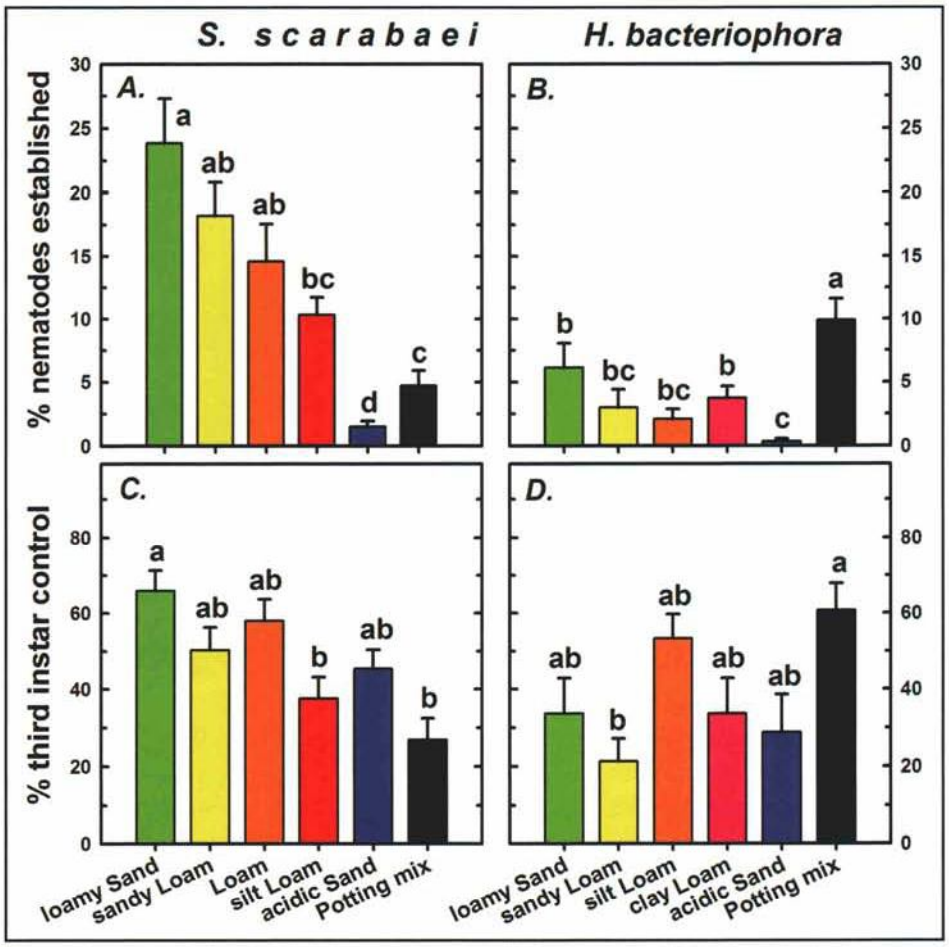

Figure 1. Effect of different substrates on infectivity (A-B) and efficacy (C-D) of the entomopathogenic nematode *Steinernema scarabaei* against the third-instar oriental beetle (A, C) and *Heterorhabditis bacteriophora* against the third-instar Japanese beetle (B, D). Columns with the same letter are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

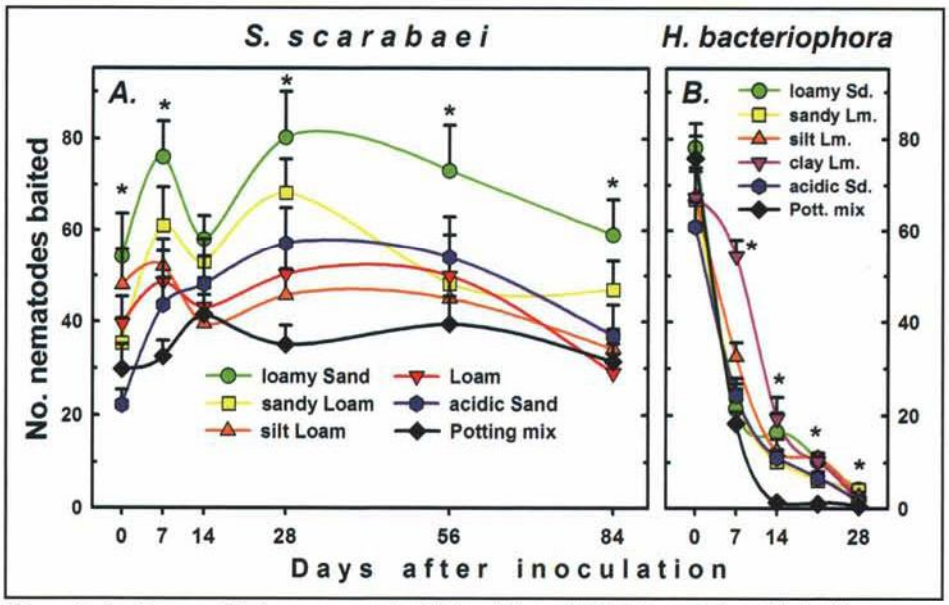

Figure 2. Persistence of *Steinernema scarabaei* (A) and *Heterorhabditis bacteriophora* (B) in different substrates. Asterisk (*) indicates significant differences in recovery among substrate types per baiting date ($P<0.05$). A significant decline in recovery was found in no substrate for *S. scarabaei*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

tested *S. scarabaei* (0 or 156 infective juveniles per pot) against oriental beetles. Experiment 2 tested *H. bacteriophora* (0 or 625 infective juveniles per pot) against Japanese beetles. The pots were destructively sampled at 14 DAT to determine the number of surviving larvae.

Mortality in the greenhouse experiment followed a trend similar to the 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 (Figure 1D).

EFFECT OF SOIL TYPE ON NEMATODE PERSISTENCE

Two laboratory experiments tested the effect of six soil types on persistence of *S. scarabaei* and *H. bacteriophora*. At different times after adding 200 infective juveniles per cup treatment (Figure 2), five cups from each treatment were opened and the soil baited with five wax moth larvae for four three-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 in all other soils, and significantly higher in sandy loam than in potting mix. *H. bacteriophora* persistence was much shorter, and recovery significantly declined in all substrates (Figure 2B). 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, -1000, -3000 kPa), allowed to equilibrate for four days, mixed again, and filled into plastic vials. Into each vial, one third-instar Japanese beetle was released and, one day later, 0 nematodes, 200 *S.*

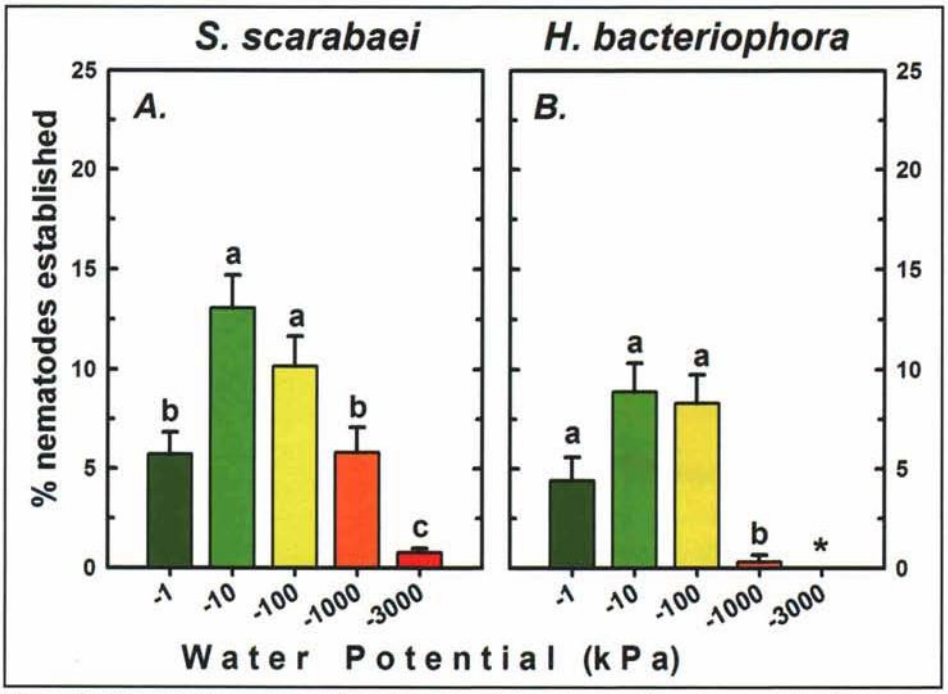

Figure 3. Effect of soil water potential on infectivity of the entomopathogenic nematodes *Steinernema scarabaei* (A) and *Heterorhabditis bacteriophora* (B) in different substrates against the third-instar *Popillia japonica*. Columns with the same letter are not significantly different ($P<0.05$).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

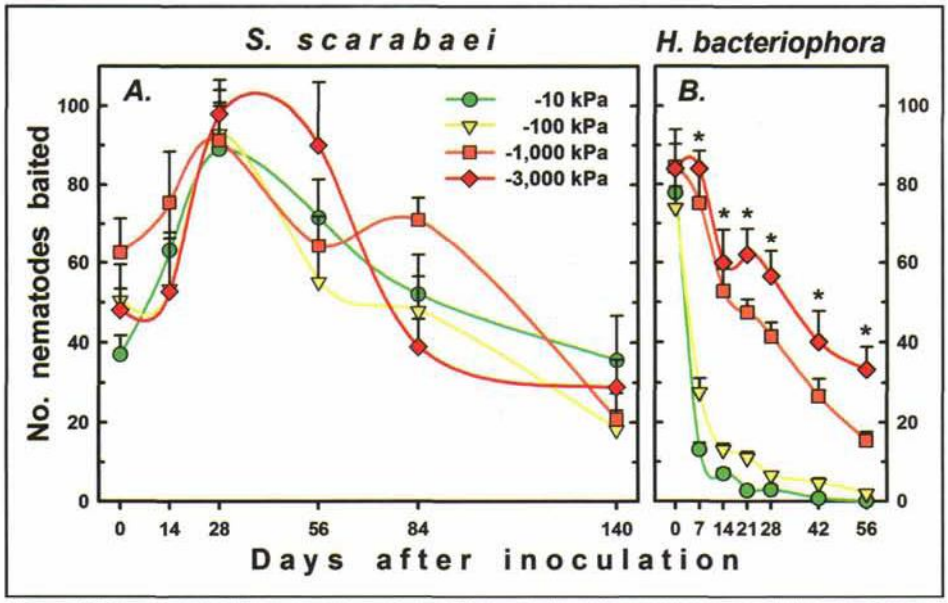

Figure 4. Persistence of *Steinernema scarabaei* or *Heterorhabditis bacteriophora* in sandy loam at four soil water potentials. Asterisk (*) indicates significant differences in recovery among water potential per baiting date (P<0.05). A significant linear decline in recovery was found for -100 kPa and -1000 kPa in *S. scarabaei* and for -10 kPa to -3000 kPa in *H. bacteriophora* (P<0.05).

scarabaei, or 1,000 *H. bacteriophora* were added to the soil. At seven days after treatment (DAT), the larvae were recovered and infected larvae were dissected and the nematodes established in them counted.

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 -3000 kPa (Figure 3A). Larval mortality by *S. scarabaei* was significantly higher at -100 kPa (100%), -10 kPa (97%), and -1000 kPa (87%), than at -3000 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 -1000 kPa and no establishment at -3000 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 -1000 kPa (17%), with no mortality at -3000 kPa.

S. scarabaei persistence was also 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 more quickly at all soil water potentials (Figure 4B), but declined the fastest at -10 kPa, somewhat slower at -100 kPa, much slower at -1000 kPa, and particularly -3000 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. Only in highly acidic sand (pH 3.9) and highly organic potting mix did *S. scarabaei* infectivity decline significantly, though it still caused significant mortality.

In comparison, *H. bacteriophora* showed similar infectivity from the coarser to the finer soils, was also 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 (-3000 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 had shorter persistence, with more useful persistence levels only in the drier soils, where it becomes inactive.

The present study indicates that long-term white grub suppression 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.

ACKNOWLEDGMENTS

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Editor's Note: This complete report can be found at USGA Turfgrass and Environmental Research Online: http://usgatero.msu.edu/v05/n19.pdf.

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CONNECTING THE DOTS

A Q&A with Dr. Albrecht Koppenhöfer, *Rutgers University, regarding the use of nematodes to control white grubs.*

Q: *In your opinion, what is the main obstacle that impedes the widespread use of entomopathogenic nematodes as a biological control of white grubs?*

A: For nematodes in general, I would think that absence of good formulations is the biggest impediment. For *Steinernema carpocapsae*, a nematode that is highly effective for control of black cutworm and other caterpillar pests, a formulation exists (wettable dispersible granules) with up to five months shelf life at room temperature. For other species and formulations, the nematodes will not last as long and often have to be kept refrigerated. What we need are formulations with tolerance to short-term temperature extremes and a shelf-life of several months at room temperature, one year and more. Nematode products also will have to become more competitive with available chemical insecticides as far as pricing and/or efficacy is concerned.

Q: *What do you believe are the most compelling reasons that future golf course superintendents should consider in using entomopathogenic nematodes for grub control?*

A: Public and legislative pressure to reduce chemical pesticide use is likely to increase in the future. In other parts of the world (e.g., Europe, Canada) and even in some parts of the USA, pesticide use is already more restricted. Nematode species that can provide long-term suppression of white grub populations would be another good reason. *Steinernema scapterisci* has already proven to effectively suppress mole cricket populations long-term, and its use is slowly gaining momentum. *Steinernema scarabaei*, should it become commercially available, could be used in a similar fashion.

Q: *Are there data from other studies to suggest the geographical range that entomopathogenic nematodes could be used to manage white grubs? Is their use restricted to less cold climates?*

A: It depends on the nematode species. *Heterorhabditis bacteriophora*, the best studied and most widely available species for grub control, should be used at soil temperatures above 68°F for maximum efficacy. This excludes spring applications and also applications after late August or even mid-August in the northern areas of the lower 48 states. *S. scarabaei*, on the other hand, has provided excellent control in late spring applications and appears to retain high activity to about 62°F. This species, however, may not do as well at soil temperatures above 85°F, and may therefore have to be applied in spring or fall in the more southern regions of the USA.

Q: *These studies involved laboratory and greenhouse experiments. How well do these results relate to field tests?*

A: These studies can only give us a general idea. Soils occur in an almost unimaginable variety, with texture, organic matter, and pH being some of the most important factors that may influence nematode performance. For example, in our experiments, *H. bacteriophora* was similarly effective across the different soil textures, but a summary of numerous field tests against Japanese beetle larvae suggested that it was more effective in the finer-textured than the coarser soils. The reason may be that finer soils retained soil moisture better and also confined the nematodes more to the upper soil layers where most of the grubs are active. Since *S. scarabaei* effectiveness declined from coarser to finer soils, it may be that it would be similarly effective across soil textures under field conditions.

Q: *The USGA has funded several research projects involving biological control of turfgrass pests. Although experiments in the lab and greenhouse may look promising, oftentimes when taken to the field, promising results cannot be repeated. What hope is there for the use of entomopathogenic nematodes as a biological control strategy?*

A: We have conducted numerous field tests in which *S. scarabaei* has provided excellent curative grub control. It has persisted in the field plots for up to four years and consistently provided significant grub control even one year after application, often also two years after application. Another very host-specific nematode, *S. scapterisci*, is providing very good long-term suppression of mole crickets in the southeastern USA. Species that are highly adapted to one of the key insect pests and provide long-term suppression may be the key to more widespread nematode use in turfgrass.

Q: *You mentioned that effective mass production technology represents the major problem in commercializing nematodes. How likely is it that effective mass production technologies will be developed?*

A: Effective mass production technologies already exist for various nematode species, including *H. bacteriophora*, *S. carpocapsae*, and *S. scapterisci*. *S. scarabaei* is difficult to mass produce. One of the companies we have collaborated with has already produced *S. scarabaei in vitro* (i.e., in media without insects), and these nematodes were as virulent and viable as the ones produced in grubs. However, production in larger quantities in liquid media has proven to be too variable. Mass production of *S. scarabaei* should be feasible, but it may take some more basic research on its nutritional requirements, and with that, more time before commercialization will be possible.

Jeff Nus, Ph.D., *manager, Green Section Research.*