Nematodes as Allies

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Nematodes are colorless, unsegmented roundworms that live in aquatic and soil environments. Their name is derived from a Greek word meaning "threadlike", which aptly describes their appearance. They commonly range in size from .3 to 2 mm in length. However, one species that parasites sperm whales is over 8 m long! The larger species found in soil are visible with the naked eye, whereas the majority of species require magnification for viewing. You have likely seen nematodes when examining a handful of soil or if you have ever dissected a fish or bird.

Nematodes inhabit a wide variety of environmental niches. Turf managers commonly think of plant parasites, but most nematodes are harmless or helpful free-living animals that feed on bacteria, fungi, algae or other nematodes. Approximately 50 percent are marine, 25 percent are free-living species found in soil or freshwater, 15 percent are parasites of animals, and 10 percent parasitize plants. The animal parasites are notorious for causing diseases such as heartworm in dogs and cats; and elephantiasis, trichinosis and river blindness in humans. However, animal parasites can be beneficial to us when insects are the host organisms. Nematodes that parasitize and cause disease in insects are referred to as "entomogenous", "entomopathogenic" or simply "beneficial". They occur naturally in soils and are also sold as pest control products.

Nematodes are the most numerous multicellular animals on earth, and their densities have been estimated from 3.5 to 9 million per m^2 of land (Sohlenius, 1980). In soil, nematodes play a major role in decomposing organic matter and recycling nutrients. Recently attention has been focused on using them as indicators of soil quality and health. Nematodes are also making a significant contribution to science in general by serving as models for studies in genetics, molecular and developmental biology.

Plant parasites

Plant parasitic nematodes are certainly not considered allies by turf managers. They have a spear-like mouthpart called a stylet that they use to penetrate plant tissue. In turfgrass, they attack root systems and feed externally or internally on root cells. Root lesions, swellings or knots often result. These nematodes directly damage plants by reducing root function, and indirectly by introducing other pathogens such as bacteria and fungi. Damage is most severe under hot conditions. The awl and sting nematodes commonly attack warm season turfgrass; whereas the dagger, spiral, stylet, ring, and lesion nematodes are problems on cool season grasses. The spiral, stubby-root, lance and root-knot nematodes affect both cool and warm season grasses. Diagnosis of plant parasitic nematode infections can be difficult, and should be verified by a plant disease diagnostic lab.

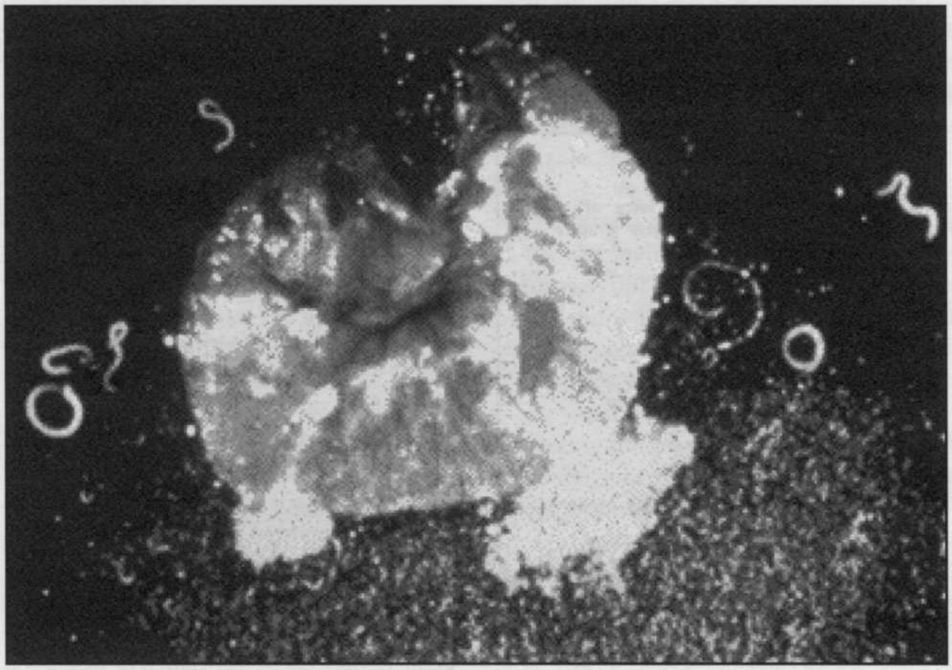
Insect parasites

Many nematodes are parasites of insects. Some large nematodes act as true parasites, which means that only one or two worms infect a host and the insect may remain healthy enough to live, feed and reproduce. However, the nematodes that have received the most attention as biological control agents act more like pathogens than parasites. This means that several nematodes invade a host, reproduce exponentially inside the insect and kill it relatively quickly. The term "**entomopathogenic**" used to describe these nematodes means "to cause disease in insects". Nematodes in the genera *Heterorhabditis* and *Steinernema* are the best studied examples and have been used commercially for several years. The story of how these worms infect and kill insects is complex and fascinating, and depends heavily on mutualistic bacteria to do the dirty work. Following is a general description of the life cycle of *Heterorhabditis* and *Steinernema* nematodes.

Nematodes exist in the soil as infective juveniles (IJs) and carry a mutualistic bacteria in their intestines. IJs come in contact with a suitable insect host and enter it through natural openings such as the mouth, anus or spiracles (breathing holes), or sometimes actively penetrate the insect's cuticle. When the nematode reaches the insect's haemocoel (body cavity), the bacteria are released and begin to rapidly multiply. The bacteria produce toxins that kill the insect host in 24-48 hours.

Nematodes and bacteria have evolved together to form a close and interdependent association. Each species of entomopathogenic nematode carries a unique species of bacteria. Heterorhabditids are associated with bacteria in the genus *Photorhabdus*, and Steinernematids are associated with *Xenorhabdus*. The bacteria perform several important functions in addition to causing rapid death of the host. They release antibiotic compounds that protect the insect cadaver from being invaded by secondary, opportunistic bacteria and fungi. The bacteria also break down and digest the host tissues to fuel their reproduction. The nematodes in turn survive by eating the bacteria as well as the broken-down insect body contents. This all seems wonderful for the nematodes, but what do the bacteria gain by the association? Nematodes provide two important services: they transport the bacteria safely through the soil from one host to the next, and they penetrate the insect and deliver the bacteria to the gut of the new host. These bacteria cannot survive on their own in soil, and have no form of locomotion. In the laboratory, nematodes can kill insects and survive without the bacteria present. However, it is unlikely that this situation is common in nature.

While bacteria are busy multiplying in the insect gut, the IJ nematodes who entered the host mature into adults, mate, and begin their own exponential reproductive process. One or two pioneering nematodes are sufficient to start a new colony, since some species are hermaphroditic. A mated female (or hermaphrodite) carries many of her eggs internally that eventually hatch inside her. These first stage juveniles molt into a second stage and then exit the mother into the body cavity of the insect cadaver. Here they normally feed, mature and produce one or two more generations, depending on food supply and crowding. The third stage juveniles from the final generation are the IJs that exit what

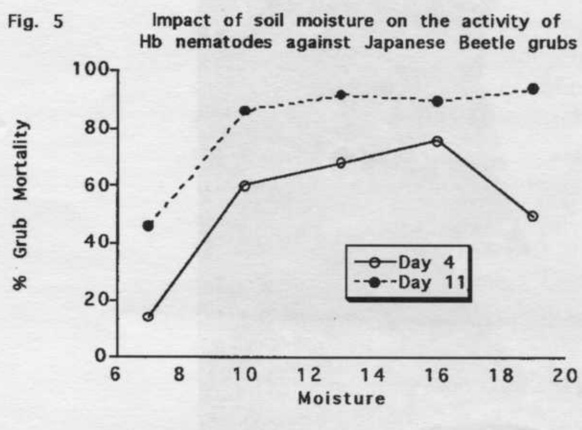


Japanese beetle grub infected with Heterorhabditis bacteriophora with reproductive nematodes visible through cuticle. Photo credits: J. Ogrodnick, NYSAES

remains of the cadaver and go out into the soil to start a new colony in another host. The IJs do not require food, and are physiologically and morphologically adapted to survive in the harsh soil environment while waiting or searching for a new host (Poinar, 1990). New IJs leave the cadaver one to two weeks after the infection is initiated.

Insects, of course, have some defenses against nematodes and their allied bacteria. For example, insect immune systems react to foreign invaders by encapsulating them or walling them off in melanin (a liquid material that hardens around the invader). Nematodes are often too large for this strategy to work, and the bacteria are protected up to the time they are released and begin rapid multiplication. Another defense used by Japanese beetles and other insects is to groom themselves with their legs to remove irritants such as nematodes on their skin (Gaugler et al., 1994). Many scarabs also have fine mesh sieve plates covering their spiracles (Gaugler et al., 1994; Wang et al., 1995), forcing nematodes to use an alternate route of entry. There are also natural mortality agents such as fungi, insects and other nematodes that attack entomopathogenic nematodes. The insect defenses and natural mortality agents can usually be overcome by inundating an area with nematodes as part of a pest management program.

Fig. 5



Impact of soil moisture on the activity of Hb nematodes against Japanese beetle grubs.

Nematode behavior

The behavior of nematodes in soil differs between species and significantly affects which insects are commonly attacked. One way of life employed by nematodes is called an "ambush" or "sit and wait" strategy. Typically, the worm is attached by its tail to a soil particle at one end while the body is extended outward waving and waiting for a host to pass by. The nematode can spring and leap off the soil particle if it senses a suitable host or wants to move to a new location. *Steinernema carpocapsae* is a commercially available nematode that exemplifies this strategy. It prefers to live in the upper 15 cm of the soil (Ferguson et al., 1995) and works best against pests that are actively moving through the thatch and soil such as cutworms and webworms.

At the other end of the spectrum of behaviors are "hunting" or "searching" nematodes. *Heterorhabditis* bacteriophora and *Steinernema glaseri* typify this strategy. These nematodes actively swim through the soil in search of hosts, and travel as deep as 35 cm deep (Ferguson et al., 1995). They are ideal agents for combating sedentary pests such as scarab grubs.

Environmental factors, especially the availability of adequate soil moisture, are critical for entomopathogenic nematode performance in the field (Georgis and Gaugler, 1991).

Because entomopathogenic nematodes need both high relative humidity to survive and a film of free water for movement, moisture conditions have been recognized as one of the most important factors in the soil environment affecting survival, infectivity and persistence of nematodes (Curran 1993, Klein 1990).

Investigations in our laboratory have shown wide variations in entomopathogenic nematode performance at varied soil moistures. For example, Figure 5 shows mortality of Japanese beetle grubs exposed to *Heterorhabditis bacteriophora* (HB) nematodes. Mortality was significantly lower in dry soil (7 percent w/w) after both 4 and 11 days. The 4 day readings also suggest that very moist soil (19 percent w/w) may inhibit nematode activity.

Another test compared mortality of Galleria (waxmoth) larvae exposed to three species of nematodes in high moisture soils (15 percent w/w) and low moisture soils (6 percent w/w). (SC = *Steinernema carpocapsae*, SF = *Steinernema feltiae*). In the high moisture treatments, efficacy of SC dropped significantly by the fifth reading (15 days after nematode inoculation); whereas the other two species continued to cause near 100 percent mortality. In the dry soil, the Steinernematid species began with high activity that dropped steadily over the first five readings. HB appears to be more sensitive to the dry conditions, and caused minimal mortality during the same time period. After the fifth reading, soil moistures in all treatments were raised to the level of the wettest treatment (hydration). This resulted in 100 percent of the insects being killed in all dry treatments—suggesting that entomopathogenic nematodes can endure long periods of dry soil conditions in a quiescent (inactive) state.

Currently, we are conducting a full year investigation to determine how long quiescent nematodes can live in dry soil and then be reactivated by the addition of moisture. We are comparing three nematodes (HB Oswego strain, HB Tuscarora strain, and SG) at four initial soil moistures ranging from very dry to very moist (6-15 percent w/w). At the most recent reading (month 7), the HB strains stored in the driest soils were 100 percent infective after rehydration. This information can aid the selection of species and strains that would survive best in irrigated and non-irrigated turf. In the future it may also be possible to apply inactive nematodes at a convenient time during the growing season, and later activate them with irrigation.

Field Applications

Many factors make nematodes an ideal microbial control agent: they have a relatively broad host range, will not attack plants or vertebrates, are easy to mass produce, and can be applied with most standard insecticide application equipment. Additionally, nematodes can search for hosts and kill them rapidly, and can release thousands of mobile progeny able to locate and infect new insect hosts within weeks of the initial infection. Because they are considered predators, entomopathogenic nematodes are exempt from registration by the United States Environmental Protection Agency (US EPA). This exemption from long term safety and water quality studies has greatly reduced the costs and risks typically associated with registering a new insecticide.

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Although there have been many successful field applications of entomopathogenic nematodes for turf insect control, problems with product quality, persistence and host specificity have led to some unsatisfactory results. Entomopathogenic nematodes have a fairly broad host ranges in laboratory studies, but different strains and species of nematodes vary in activity against insect species in the field.

In the mid 1980's, several species of entomopathogenic nematodes became commercially available for insect pest management. Initially, small-scale production and limited marketing resulted in these products being used mainly for home gardens, lawns and landscapes. More recently, a few large companies have attempted mainstream marketing aimed at the commercial turf, vegetable, and fruit industries, but acceptance has been hindered by variability in the success of the nematodes' ability to control target insects.

The preceding information on nematode biology and behavior provides a good base for discussing the practical aspects of using entomopathogenic nematodes for management of insect pests. Many previous unsuccessful field applications of entomopathogenic nematodes for scarab grub control can be traced to inappropriate conditions at the time of application and for several weeks post application (Villani et al. 1992). Turf managers can get more consistent results by following these suggestions. First and foremost, turf managers should follow the basic principles of good pest management. This means you need to know the identity, biology, location and density of your target pests, which will require scouting and monitoring. The next step is to determine if any commercially available beneficial nematodes are known to attack your pests.

Another consideration is the life stage of your pest species. Nematodes are usually most effective against the early stages of larval insects. Eggs, large larvae, pupae and adults are often difficult or impossible to infect. One exception is *S. scapterisci* that infects adult mole crickets as they tunnel in the soil. If you are in doubt about the timing of a nematode application, consult the supplier or your local Cooperative Extension agent.

Nematodes can be applied with most standard pesticide application equipment if the psi does not exceed 300, and the screen mesh is no less than 50 microns. Because the material is live, users must be very careful to store it in a cool place and not exceed the recommended shelf life. The nematodes must also be agitated while in the spray tank to ensure uniform distribution and to circulate oxygen to them. The suggested application rate is one billion nematodes per acre for most soil insects. Higher rates can be used when drenching ornamental plantings.

Your turf management practices before, during and after a nematode application impact its effectiveness. Turf should be irrigated before and after an application to prevent nematodes from drying, sticking and dying on the grass blades. The heaviest nematode mortality occurs in the first few minutes and hours after application (Smits, 1996), so this time period is critical. The post application irrigation will also help wash the nematodes down through the turf and thatch layers. Be sure the turf remains well irrigated during the subsequent weeks, since nematodes need a moist environment to be infective, as discussed previously. Chemical pesticides applied shortly before or after the nematode application may lessen effectiveness, since nematodes are often sensitive to them. The following turf products have been shown to negatively impact *H. bacteriophora* nematodes: bendiocarb, chlorpyrifos, and ethoprop, the fungicides anilazine, dimethyl benzyl ammonium chloride, fenarimol, and mercurous chloride, the herbicides 2,4-D and trichlopyr, and the nematicide fenamiphos (Rovesti et al., 1988).

Another basic pest management principle to follow is to monitor the pest population after the nematode application to evaluate its effectiveness. It may take a few days for nematodes to locate a host, but the insect will die in 24-48 hours after a successful infection. Infected insects have a characteristic, uniform color because of the bacteria present. *Heterorhabditis-Photorhabdus* infections produce red or purple cadavers and the bacteria actually glow in the dark! *Steinernema-Xenorhabdus* cadavers are tan or beige. The cadavers are flaccid, intact, and typically do not show signs of

TABLE 1. TURFGRASS INSECTS ATTACKED BY NEMATODES

Target Pest	Nematode Species
Scarab grubs, black vine weevils	*Heterohabditis bacteriophora*
Mole crickets	*Steinernema riobravis, S. scapterisci*
Billbugs	*H. bacteriophora, S. carpocapsae*
Armyworm, Cutworm, Webworm, Annual Bluegrass Weevil*	*S. carpocapsae*
Plant pathogenic nematodes (possibly)	*S. riobravis*

**some success reported*

TABLE 2. ENTOMOPATHOGENIC NEMATODE SUPPLIERS

Product name	Nematode Species	Supplier or Producer	Telephone
Heteromask	Hb (Oswego)	IPM Laboratories	(315)-497-2063
Grub Away	Hb	Gardens Alive	(812)-537-8650
Lawn Patrol	Hb	Hydro-Gardens	(800)-634-6362
Beneficial Nematode	Hb (Merilatus & HP88)	M & R Durango	(800)-526-4075
Grub Guard	Hb & Sf	North Country Organics	(802)-222-4277
Guardian	Sc	Hydro-Gardens	(800)-634-6362
Ecomask	Sc	IPM Laboratories	(315)-497-2063
Beneficial Nematodes	Sc (All & Kapow)	M & R Durango	(800)-526-4075
Combo (Lawn Patrol + Guardian)	Hb & Sc	Hydro-Gardens	(800)-634-6362
Scanmask	Sf	IPM Laboratories	(315)-497-2063
Beneficial Nematodes	Sf	Gardens Alive	(812)-537-8650

Nematode species
Hb = *Heterorhabditis bacteriophora*, Sc = *Steinernema carpocapsae*, Sf = *Steinernema feltiae*

other infections (e.g. fungus) or rotting. Live nematodes can be seen through the cuticle of some insects. When monitoring, keep track of the number of healthy and infected insects detected.

Entomopathogenic nematodes can be introduced or conserved from local natural populations in turfgrass plantings, to help keep insect populations in check. Remembering the ecological and management principles outlined here will help in accomplishing that goal. The next time you see a dead or dying insect on a lawn or golf course, take a moment to try and figure out if our small friend the nematode played a role.

In Future Issues

- Mechanisms of disease resistance
- Endophytes in turf