Using Entomopathogenic Nematodes for Crop Insect Pest Control

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Using Entomopathogenic Nematodes for Crop Insect Pest Control

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Introduction

Nematodes are non-segmented, elongated roundworms that are colorless, without appendages, and usually microscopic. There are non-beneficial and beneficial nematodes. Non-beneficial nematodes are also called "plant parasitic nematodes" and cause damage to crops and other types of plants. Beneficial nematodes attack soilborne insect pests, yet are not harmful to humans, animals, plants, or earthworms, and can therefore be used as biological control organisms (Denno et al. 2008). Beneficial nematodes that cause disease within an insect are referred to as "entomopathogenic" and have the ability to kill insects (Figure 1).

Entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae have proven to be the most effective as biological control organisms (Kaya and Gaugler 1993). They are soil-inhabiting organisms and can be used effectively to control soilborne insect pests, but are generally not effective when applied to control insects in the leaf canopy. When considered as a group of nearly 30 species, each with its own suite of preferred hosts, entomopathogenic nematodes can be used to control a wide range of insect pests, including a variety of caterpillars, cutworms, crown borers, grubs, corn root worm, cranefly, thrips, fungus gnat, and beetles. Entomopathogenic nematodes have been released extensively in crop fields with negligible effects on nontarget insects and are regarded as exceptionally safe to the environment.

The keys to success with entomopathogenic nematodes are (1) understanding their life cycles and functions; (2) matching the correct nematode species with the pest species; (3) applying them during appropriate environmental conditions (soil temperature, soil moisture, sunlight); and (5) applying them only with compatible pesticides. Because entomopathogenic nematodes are living organisms, they require careful handling to survive shipment and storage as well as appropriate environmental conditions to survive in the soil after application.

Many species of entomopathogenic nematodes occur naturally in the Pacific Northwest, including *Heterorhabditis marelata*, which has been shown to control some insect crop pests (Berry et al. 1997). However, most entomopathogenic nematodes used for pest management are not native to the region. It is unknown whether these introduced species will overwinter in the Pacific Northwest and successfully colonize insect hosts in subsequent years. While much of the research regarding entomopathogenic nematodes has been conducted outside of the region, the information in this publication can be successfully applied throughout the Pacific Northwest.

Life Cycle of Entomopathogenic Nematodes

The life cycle of most nematodes includes an egg stage, four juvenile stages, and an adult stage. The third juvenile stage of entomopathogenic nematodes is referred to as the "infective juvenile" or "dauer" stage and is the only free-living stage (Figure 2). The infective juvenile is capable of surviving in the soil, where it locates, attacks, and infects a pest insect (Poinar 1990). Under optimal conditions, it takes 3–7 days for steinernematids and heterorhabditids to complete one life cycle inside a host from egg to egg. Emergence of infective juveniles from the host requires about 6–11 days for steinernematids and 12–14 days for heterorhabditids (Kaya and Koppenhöfer 1999). Figure 3 is a diagram of the life cycle of entomopathogenic nematodes from host infection to emergence from the host.

Figure 1. Tens of thousands of infective juveniles of the entomopathogenic nematode Steinernema carpocapsae *spilling out of a Galleria wax moth larva in search of new hosts (photo provided by Randy Gaugler).*

How Entomopathogenic Nematodes Control Insect Pests

An understanding of host-finding strategies will help you properly match entomopathogenic nematode species to pest insects to ensure infection and control (Gaugler 1999). Only entomopathogenic nematodes in the infective juvenile stage will survive in the soil and find and penetrate insect pests. Infective juvenile entomopathogenic nematodes locate their hosts in soil

Figure 2. An infective juvenile entomopathogenic nematode (photo provided by Randy Gaugler).

by means of two strategies—ambushing and cruising (Gaugler et al. 1989). Ambusher species include *Steinernema carpocapsae* and *S. scapterisici*; cruisers include *Heterorhabditis bacteriophora* and *S. glaseri*. *S. riobrave* and *S. feltiae* do a bit of both ambushing and cruising (Campbell and Gaugler 1997).

Ambushing. Entomopathogenic nematodes that use the ambushing strategy tend to remain stationary at or near the soil surface and locate host insects by direct contact (Campbell et al. 1996). An ambusher searches by standing on its tail so that most of its body is in the air, referred to as "nictation." The nictating nematode attaches to and attacks passing insect hosts. Ambusher entomopathogenic nematodes most effectively control insect pests that are highly mobile at the soil surface, such as cutworms, armyworms, and mole crickets.

Cruising. Entomopathogenic nematodes that use the cruising strategy are highly mobile and able to move throughout the soil profile. Cruisers locate their host by sensing carbon dioxide or other volatiles released by the host. Cruiser entomopathogenic nematodes are most effective against sedentary and slow-moving insect pests at various soil depths, such as white grubs and root weevils.

Infection

Generally, several entomopathogenic nematodes will infect a single insect host. Infective juvenile nematodes penetrate the insect's body cavity either through natural body openings (such as the mouth, anus, genital pore, or breathing pore; Figure 4) or by breaking the outer cuticle of the insect; heterorhabditids do this using a dorsal "tooth" or hook. Once inside the body cavity of

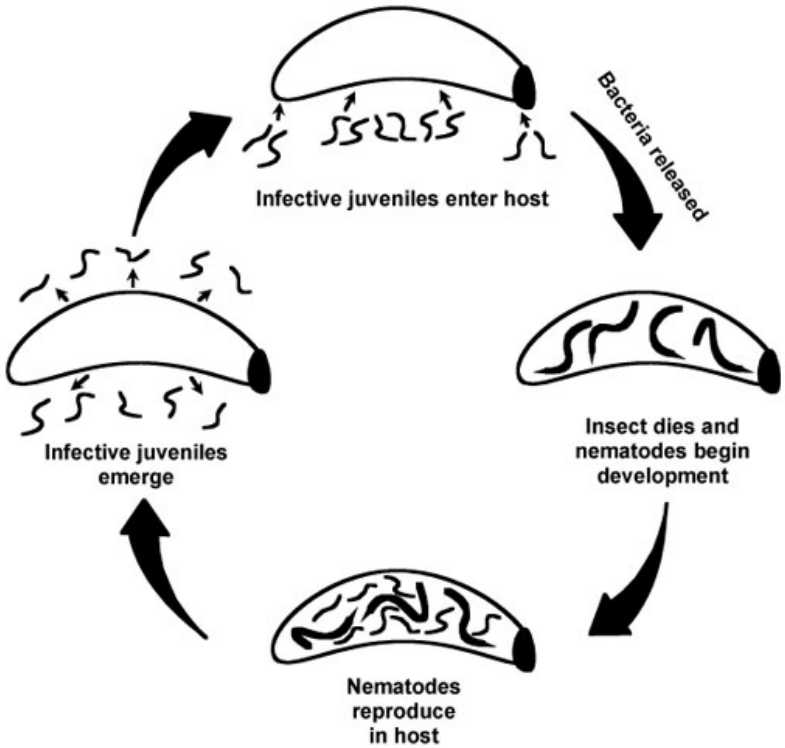

Figure 3. Diagram of the generalized life cycle of entomopathogenic nematodes (image provided by David Shapiro-Ilan).

the host, the infective juveniles release bacteria that live symbiotically within the entomopathogenic nematode's gut but do not harm the nematode. The nematode-bacterium relationship is highly specific: only *Xenorhabdus* spp. bacteria co-exist with steinernematids, and only *Photorhabdus* bacteria co-exist with heterorhabditids. Once released into the host, the bacteria multiply quickly and under optimal conditions cause the host to die within 24 to 48 hours.

Entomopathogenic nematodes feed on both bacteria they release and host insect tissue. After a few days inside the host, entomopathogenic nematodes mature to the adult stage. These adult entomopathogenic nematodes produce hundreds of thousands of new juveniles that may undergo several life cycles within a single host (Figure 5). When the host has been consumed, the infective juveniles, armed with a fresh supply of bacteria, emerge from the empty shell of the host, move into the soil, and begin the search for a new host. A protective exterior cuticle surrounds the infective juvenile, protecting it from the environment and predators. Under ideal conditions, steinernematids emerge 6–11 days after initial infection and heterorhabditids emerge 12–14 days after initial infection (Kaya and Koppenhöfer 1999). The duration of infective juvenile survival in soil is unknown because they can become prey to invertebrates and microorganisms.

Using Entomopathogenic Nematodes

Worldwide, over 80 species of entomopathogenic nematodes have been identified and 11 commercialized (Kaya and Koppenhöfer 1999). The different species of entomopathogenic nematodes vary in the range of insects they attack, environmental needs, and stability in commercial products (Gaugler 1999). A given species of entomopathogenic nematode may also control a particular pest more effectively than another species. Therefore, the insect pest must be identified before choosing the entomopathogenic nematode species most appropriate for biological control. Table 1 is a general guide of available entomopathogenic nematodes and the pests they have been shown to control. Table 2 provides a description of each species of commercially available entomopathogenic nematode.

Obtaining Entomopathogenic Nematodes

Perhaps the biggest challenge to the use of entomopathogenic nematodes as effective biological control organisms is the variable quantity and quality of nematodes in commercial products (Gaugler et al. 2000). Entomopathogenic nematodes are cultured on a large scale in laboratories and are available from many commercial suppliers in North America and Europe. In past assessments of cottage industry commercial products, most contained lower numbers of entomopathogenic nematodes than the suppliers claimed. In addition, in some cases the species of entomopathogenic nematodes in the product were mixed and therefore inconsistent with the product label. The industry has made progress, however, in increasing the quality of its products. See Table 3 for lists of commercial suppliers compiled by university and state government professionals. Entomopathogenic nematodes can also be purchased through gardening mail-order catalogs and at some local agricultural and nursery supply stores.

Shelf Life

In general, entomopathogenic nematodes do not have a long shelf life. Many microbial insecticides, including *Bacillus thuringiensis*, have a resting stage facilitating long-term storage. The infective juvenile entomopathogenic

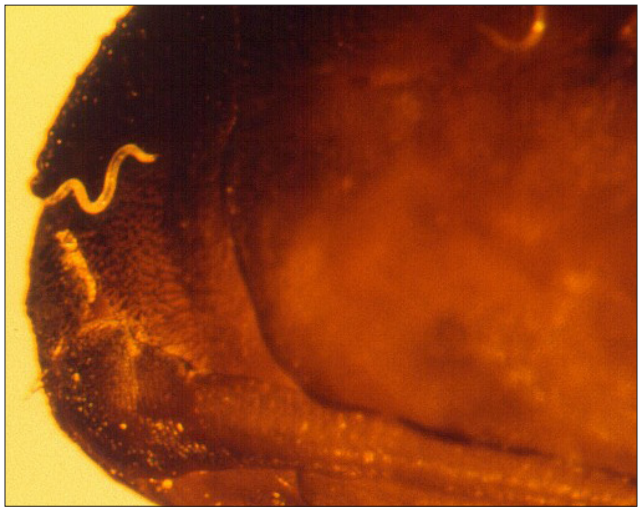

Figure 4. Infective juvenile entomopathogenic nematodes searching for an entry portal into a host (photo provided by Randy Gaugler).

Figure 5. Hundreds of thousands of new entomopathogenic nematodes within a host insect (photo provided by Randy Gaugler).

Table 1. Current use of steinernema and heterorhabditis nematodes as biological control organisms[1] (Shapiro-Ilan and Gaugler 2010).

Crop(s) Targeted	Pest Common Name	Pest Scientific Name	Efficacious Nematodes[2]
Artichokes	Artichoke plume moth	*Platyptilia carduidactyla*	Sc
Vegetables	Armyworm	Lepidoptera: Noctuidae	Sc, Sf, Sr
Ornamentals	Banana moth	*Opogona sachari*	Hb, Sc
Bananas	Banana root borer	*Cosmopolites sordidus*	Sc, Sf, Sg
Turf	Billbug	*Sphenophorus* spp. (Coleoptera: Curculionidae)	Hb,Sc
Turf, vegetables	Black cutworm	*Agrotis ipsilon*	Sc
Berries, ornamentals	Black vine weevil	*Otiorhynchus sulcatus*	Hb, Hd, Hm, Hmeg, Sc, Sg
Fruit trees, ornamentals	Borer	*Synanthedon* spp. and other sesiids	Hb, Sc, Sf
Home yard, turf	Cat flea	*Ctenocephalides felis*	Sc
Citrus, ornamentals	Citrus root weevil	*Pachnaeus* spp. (Coleoptera: Curculionidae)	Sr, Hb
Pome fruit	Codling moth	*Cydia pomonella*	Sc, Sf
Vegetables	Corn earworm	*Helicoverpa zea*	Sc, Sf, Sr
Vegetables	Corn rootworm	*Diabrotica* spp.	Hb, Sc
Cranberries	Cranberry girdler	*Chrysoteuchia* topiaria	Sc
Turf	Crane fly	Diptera: Tipulidae	Sc
Citrus, ornamentals	Diaprepes root weevil	*Diaprepes abbreviatus*	Hb, Sr
Mushrooms	Fungus gnat	Diptera: Sciaridae	Sf, Hb
Grapes	Grape root borer	*Vitacea polistiformis*	Hz, Hb
Iris	Iris borer	*Macronoctua onusta*	Hb, Sc
Forest plantings	Large pine weevil	*Hylobius albietis*	Hd, Sc
Vegetables, ornamentals	Leafminer	*Liriomyza* spp. (Diptera: Agromyzidae)	Sc, Sf
Turf	Mole cricket	*Scapteriscus* spp.	Sc, Sr, Sscap
Nut and fruit trees	Navel orangeworm	*Amyelois transitella*	Sc
Fruit trees	Plum curculio	*Conotrachelus nenuphar*	Sr
Turf, ornamentals	Scarab grub[3]	Coleoptera: Scarabaeidae	Hb, Sc, Sg, Ss, Hz
Ornamentals	Shore fly	*Scatella* spp.	Sc, Sf
Berries	Strawberry root weevil	*Otiorhynchus ovatus*	Hm
Bee hives	Small hive beetle	*Aethina tumida*	Hi, Sr
Sweet potato	Sweetpotato weevil	*Cylas formicarius*	Hb, Sc, Sf

[1] Nematodes listed provided at least 75% suppression of these pests in field or greenhouse experiments.
[2] Nematode species are abbreviated as follows: Hb = *Heterorhabditis bacteriophora*, Hd = *H. downesi*, Hi = *H. indica*, Hm = *H. marelata*, Hmeg = *H. megidis*, Hz = *H. zealandica*, Sc = *Steinernema carpocapsae*, Sf = *S. feltiae*, Sg = *S. glaseri*, Sk = *S. kushidai*, Sr = *S. riobrave*, Sscap = *S. scapterisci*, Ss = *S. scarabaei*.
[3] Efficacy against various pest species within this group varies among nematode species.

Table 2. Characteristics of seven species of entomopathogenic nematodes that are commercially available in the United States (Gaugler 1999 and Berry 2000).

Steinernema carpocapsae. An ambusher type, it is most effective against highly mobile surface insects such as webworms, cutworms, armyworms, girdlers, and wood borers. Most effective at soil surface temperatures between 70°F and 85°F. Can be formulated in a partially desiccated state in clay granules to provide several months of room-temperature shelf life.
S. feltiae. Combines ambusher and cruiser strategies and attacks immature fly larvae (dipterous insects), including mushroom flies, fungus gnats, and crane flies. Maintains infectivity at low soil temperatures, even below 50°F. Has relatively low stability in formulation and a short shelf life.
S. glaseri. A large cruiser type that attacks white grubs and other beetle larvae, particularly scarabs. Expensive and difficult to produce and manage due to its tendency to lose its bacterial symbiont. Highly active and robust infective juveniles are difficult to contain within formulations.
S. riobrave. Combines ambusher and cruiser strategies, and attacks corn earworms, citrus root weevils, pink bollworms, and mole crickets. Isolated from the Rio Grande Valley of Texas. Maintains infectivity at soil temperatures above 95°F and in semi-arid conditions.
Heterorhabditis bacteriophora. A cruiser-type nematode that attacks caterpillar and beetle larvae, including root weevils, particularly black vine weevil. Most effective in warm temperatures (above 68°F). Infective juveniles persist only a few days in the soil. Has poor stability in formulation and a short shelf life.
H. marelata. A cruiser-type nematode that attacks beetle larvae, including white grubs and root weevils. Isolated from the Oregon coastal region. Active at cool soil temperatures (50–55°F).
H. megidis. A large cruiser-type nematode that has been effective in controlling black vine weevil larvae. Has not been widely researched or tested for insect control. Isolated in Ohio, researched and developed in Europe, and now available in the United States. Tends to have poor formulation stability and a short shelf life.

Table 3. Recommended guides to commercial suppliers of entomopathogenic nematodes.

Commercial Sources of Insect Parasitic Nematodes. 2000. Compiled by P. Grewal and K. Power. Ohio State University. http://www2.oardc.ohio-state.edu/nematodes/nematode_suppliers.htm
Commercial Producers and Suppliers of Nematodes. 2010. Compiled by D.I. Shapiro-Ilan and R. Gaugler. Cornell University. http://www.biocontrol.entomology.cornell.edu/pathogens/nematodes.html
Vendors of Beneficial Organisms in North America. 2010. Compiled by J. White and D. Johnson. University of Kentucky College of Agriculture. http://www.ca.uky.edu/entomology/entfacts/ef125.asp
Suppliers of Beneficial Organisms in North America. 1997. Compiled by C.D. Hunter. California Environmental Protection Agency, Department of Pesticide Regulation, Environmental Monitoring and Pest Management Branch. http://www.cdpr.ca.gov/docs/pestmgt/ipminov/bensup.pdf

nematode stage is not a resting stage; juveniles are metabolically active and use energy reserves while in formulation (Lewis 1999). For this reason, it is advisable to order entomopathogenic nematodes only 3–4 days prior to application. Entomopathogenic nematodes should be shipped by overnight delivery in their infective juvenile stage and used within 1–2 days after arrival.

Examine the entomopathogenic nematodes upon receipt to make sure they arrived alive. The shipment container should not feel warm or hot. Open the container and check the color and odor of the nematodes. To the naked eye, the nematodes on a sponge formulation will appear as a light tan or gray paste, while nematodes in vermiculite or liquid suspension will not be discernible from the carrier material. The container should have a mild odor; if there is a strong smell, like ammonia, then it is likely the nematodes are dead. If the formulation is a sponge or vermiculite, remove a tiny portion of the product with tweezers and place in a teaspoon of cool water (approximately 60ºF) for six hours. If the formulation is a liquid suspension, swirl the liquid to ensure distribution of the nematodes and remove a small droplet (about 0.05 ml).

Place the soaked nematode sample (from the sponge or vermiculite) or the droplet from the liquid suspension on a slide or in a small, clear glass bowl. View the samples with a hand lens (15X) or microscope. Live entomopathogenic nematodes will be mobile and have a bend to their shape. *S. carpocapsae* has a resting "J" shape and will move only when prodded with a pin or needle (Figure 2). All other nematodes will move in an "S" pattern (Lewis 1999). If the nematodes are straight and not moving, it is likely they are dead. A mortality rate of 10% is typical. If more than 20% of the nematodes are dead, inform your supplier immediately.

Entomopathogenic nematodes should be stored in their shipment containers under refrigeration until ready for use. The storage life of entomopathogenic nematodes is species- and formulation-dependent. Specific storage instructions will be included with the entomopathogenic nematode shipment and should be carefully followed. Table 4 is a summary of storage times for entomopathogenic nematodes in different formulations. Storing nematodes under refrigeration will increase their shelf life, but their infectivity will still decrease the longer they are in storage. When the

Table 4. Expected storability of some commercially available formulations of entomopathogenic nematodes (adapted from Grewal 1999b).

		Storage Duration	
Formulation	**Nematode Species**	**Room Temperature**	**Refrigerated**
Liquid concentrate	*S. carpocapsae* *S. riobrave*	5–6 days 3–4 days	12–15 days 7–9 days
Sponge	*S. carpocapsae* *H. bacteriophora*	0 days 0 days	2–3 months 1–2 months
Vermiculite	*S. feltiae* *H. megidis*	0 days 0 days	4–5 months 2–3 months
Alginate gels	*S. carpocapsae* *S. feltiae*	3–4 months 1/2–1 month	6–9 months 4–5 months
Flowable gels	*S. carpocapsae* *S. glaseri*	1-1 1/2 months 0 days	3–5 months 1–1 1/2 months
Water dispersible granules	*S. carpocapsae* *S. feltiae* *S. riobrave*	4–5 months 11/2–2 months 2–3 months	9–12 months 5–7 months 4–5 months
Wettable powder	*S. carpocapsae* *S. feltiae* *H. megidis*	2 1/2–3 1/2 months 2–3 months 2–3 months	6–8 months 5–6 months 4–5 months
Nematode wool	*H. bacteriophora*	21 days	unknown

storage life has expired, expect 70–100% mortality of the nematodes (Grewal 2000).

Soil Conditions

Entomopathogenic nematodes can die if they are applied to soils that are too dry, too hot, or too cold, or if they are exposed to ultraviolet (UV) light from the sun. Nematodes live in the water-filled spaces, or pores, between soil particles. They need water to move and successfully locate a host, and oxygen to survive. Heavy clay soils hold water well, but may contain too little oxygen, and the small pore space may restrict nematode movement. Sandy soils must be irrigated to maintain the water-filled pores. If applying entomopathogenic nematodes after an extended dry period, break the crust of the dry soil with a rake or harrow and irrigate the soil before the application, preferably to a depth of 4–6 inches. Remove plant debris before the application, as it will prevent the nematodes from reaching the soil surface.

Soil temperatures between 77°F and 82°F are ideal for applying all entomopathogenic nematode species. In general, soil temperatures greater than 85°F can decrease the efficacy of some nematode species, while soil temperatures less than 50°F can immobilize others at the soil surface, causing them to be exposed to UV light that can kill them. The range of soil temperatures that nematode species can survive and infect host insects does vary, however. For example, *S. feltiae* can be effective at 57°F, while *S. riobrave* can be effective at 95°F.

Entomopathogenic nematodes should be applied late in the day or on a cool, overcast day when light and temperatures are low. Apply them to moist soil following either a rainfall or irrigation, and lightly irrigate afterward. This washes them into the soil and decreases soil surface temperatures. Do not over-irrigate, as saturated soil will impede nematode activity due to lack of oxygen.

Preparing for Application

Entomopathogenic nematodes should be prepared for field application no earlier than one hour ahead of time. If nematodes are in a liquid suspension, shake the shipment container well and pour the liquid into the application container (e.g., tank, backpack sprayer, or watering can). Rinse the shipment container twice with cool water (approximately 60°F), and pour the rinse water into the application container. If nematodes are on a sponge, soak the sponge in one gallon of cool water for 10 minutes, then pour the water into the application container. Rinse the sponge several times, pouring the rinse water into the application container after each rinse. If nematodes are in vermiculite, add the vermiculite-nematode mixture directly to water in the application container and stir until dispersed. Once the nematodes have been mixed with water, agitate the mixture every five minutes to keep the nematodes in suspension and supplied with oxygen.

Application Equipment

Read the product label for specific application instructions. Entomopathogenic nematodes that are formulated

with vermiculite may be best applied as a granular product. Other formulations can be applied using standard liquid pesticide, fertilizer, and irrigation equipment with pressures of up to 300 PSI. Electrostatic, fan, pressurized, and mist sprayers can be used. If tanks are agitated through excessive sparging (recirculation of the spray mix), or if the temperature in the tank rises above 86°F, the nematodes will be damaged. Irrigation systems may also be used for applying most species; however, high-pressure recycling pumping systems are not good delivery systems (Shetlar 1999). An excellent overview of sprayer equipment is provided in the *Private Applicator Pesticide Education Manual,* EM020 (Ramsay et al. 2009).

Remove all screens smaller than 50-mesh from your spray or irrigation equipment to allow nematodes to pass through the system. Check spray nozzle orifices for clogging during application. Direct spray nozzles at the soil to maximize the number of nematodes being applied directly to the soil. A large spray volume is ideal. Volumes of 2–6 gallons of water per 1,000 square feet (87–260 gallons per acre) are recommended on most nematode labels (Shetlar 1999). The water in the spray will wash the nematodes from plant surfaces into the soil. Lower volume spray applications of 0.5–1.0 gallon per 1,000 square feet (20–45 gallons per acre) can be used if the area or field is irrigated prior to and immediately following entomopathogenic nematode application. Overhead irrigation following nematode application will wash the nematodes from plant surfaces into the soil. If the spray droplets are allowed to dry prior to this irrigation, the nematodes will be exposed to UV light and die while still on the plant surfaces. Applying nematodes during a rainfall will also ensure that nematodes reach the soil surface.

Use equipment that is clean and free of pesticide residues. Also, do not mix entomopathogenic nematodes with nitrogen fertilizers, particularly urea (Grewal 2000). Although there is evidence that nematodes are tolerant to many herbicides and fungicides, they are sensitive to certain insecticides and nematicides. Refer to the nematode product label for specific listings of chemicals that are lethal to entomopathogenic nematodes. To check that live nematodes are being applied to the soil, set pans or containers on the soil surface prior to application. Immediately after application, use a hand lens (15X) or a microscope to check the liquid in the pans or containers for live, moving nematodes.

Application Rates

Before applying any biological control, including entomopathogenic nematodes, read the product label for specific application instructions. A broadcast application rate of 1 billion nematodes per acre is generally recommended to control most soil insects. For smaller areas, the recommended application rate is 250,000 nematodes per square meter. If nematodes are banded (applied in a band beside the crop row), a lower rate may be applied. Research at the University of Florida has demonstrated that a rate of up to 200 million nematodes per acre applied in a band provided effective control of root weevil in citrus orchards (Duncan et al. 1999). More research is needed to determine specific rate responses for each species of entomopathogenic nematode in various cropping systems to control specific pests. Calibrate equipment to ensure appropriate application rates. An excellent overview of sprayer calibration is provided in the *Private Applicator Pesticide Education Manual,* EM020 (Ramsay et al. 2009).

Evaluating nematode applications

It can be difficult to be sure if the entomopathogenic nematodes reached the soil and the target pests, as it is very laborious to recover the cadavers of the insects they have killed. There are two simple tests that can be used to assess the efficacy of all entomopathogenic nematode species. Both tests employ *Galleria mellonella* waxworms (Berry 2007), which are the caterpillar stage of a waxmoth species that are extremely susceptible to entomopathogenic nematode infection. *Galleria* waxworms are readily available at fishing bait and pet supply stores.

For the first test, place 2–3 *Galleria* waxworms in a tea strainer and bury the strainer 4 inches deep in the soil. You can bury the waxworms either just before you apply the entomopathogenic nematodes or anytime afterwards. It is best to place several baited strainers in the area where nematodes are being applied—either 3–4 strainers for a garden area or approximately 10 strainers per acre. Remove the strainers from the soil after 2 days, rinse the waxworms with distilled water, and store them on moistened filter paper or thick paper towel in a dark location at room temperature. Check the waxworms regularly over the next 7–10 days to look for nematode infection. Infected waxworms will usually change color; Steinernematid-infected waxworms will turn yellow, tan, or brown, while heterorhabditid-infected waxworms will turn pink or purple. If the waxworms turn black, they were likely killed by other means.

For the second test, collect entomopathogenic nematode-treated soil from the treated area at least one day after nematodes have been applied. Collect at least 10 soil samples from a garden area and 20 soil samples per acre. Each soil sample should be approximately ¼ cup from a depth of 4 inches. Mix the soil together, place ¼ cup into a wax cup, and place a Galleria waxworm on top of the soil. Evaluate 2–3 wax cups for a garden area or approximately 10 wax cups per acre. Place the cups in a dark area at room temperature for 2 days. Rinse, store, and evaluate the waxworms as described above.

Pesticide Safety

Entomopathogenic nematodes, like all biological control organisms, are considered pesticides. How-

ever, entomopathogenic nematodes are exempted from federal pesticide registration requirements under 40CFR152.20(a) because they belong to a group of specific biocontrol agents. Questions regarding pesticide registration should be directed to your state's Department of Agriculture Pesticide Division.

- **Washington State Department of Agriculture Pesticide Management Division**
  PO Box 42589, Olympia, WA 98504-2589
  Phone: 1-877-301-4555, Fax: (360) 902-2093,
  Email: pestreg@agr.wa.gov
  http://agr.wa.gov/PestFert/

- **Oregon State Department of Agriculture Pesticides Division**
  635 Capitol Street NE, Salem, OR 97301-2532
  Phone: (503) 986-4635, Fax: (503) 986-4735,
  Email: pestx@oda.state.or.us
  http://www.oregon.gov/ODA/PEST/index.shtml

- **Idaho State Department of Agriculture Division of Agricultural Resources Pesticide Division**
  PO Box 790, Boise, ID 83701-0790
  Phone: (208) 332-8500, Fax: (208) 334-2170,
  Email: info@agri.idaho.gov
  http://www.agri.state.id.us/Categories/Pesticides/indexPesticides.php

The environmental benefits of using entomopathogenic nematodes include no concern with re-entry times, residues, groundwater contamination, or pollinators. Any pesticide, including biological controls, applied to certified organic farms must be approved by the organic certification program. A list of approved organic materials is available from your state's Department of Agriculture Organic Program.

- **Washington State Department of Agriculture Organic Food Program**
  PO Box 42560, Olympia, WA 98504-2560
  Phone: (360) 902-1805, Fax: (360) 902-2087,
  Email: organic@agr.wa.gov http://agr.wa.gov/foodanimal/organic/

- **Oregon State Department of Agriculture Food Safety Division**
  635 Capitol Street NE, Salem, OR 97031-2532
  Phone: (503) 986-4550, Fax: (503) 986-4747,
  Email: info@oda.state.or.us
  http://www.oregon.gov/ODA/FSD/index.shtm

- **Idaho State Department of Agriculture Organic Program**
  PO Box 790, Boise, ID 83701
  Phone: (208) 332-8675, Fax: (208) 334-2170,
  Email: Brandon.Lamb@agri.idaho.gov
  http://www.agri.idaho.gov/Categories/PlantsInsects/Organic/indexOrganicHome.php

More Information about Entomopathogenic Nematodes

Insect Parasitic Nematodes—Ohio State University

http://www2.oardc.ohio-state.edu/nematodes

Provides a comprehensive bibliography of research literature that permits quick access to all published papers, particularly field trials, for any target insect. Includes an electronic expert panel of entomopathogenic nematode authorities who will respond to questions.

Plant and Insect Parasitic Nematodes—University of Nebraska

http://nematode.unl.edu/

Includes discussion of the species *Steinernema* and *Heterorhabditis*, including culture, storage, transport, and images. Includes an electronic copy of the paper.

Biological Control News—University of Wisconsin

http://www.entomology.wisc.edu/mbcn/

Provides back issues of the newsletter *Biological Control News*. Also includes an alphabetical index of biological control-related articles. For example, clicking on the "N" button will provide a list of topics on nabid bug, National Academy of Science Report, *Nealiotus curculionis*, and nematodes. Click on any of the topics for a complete article listing.

Literature Cited

Berry, R. 2007. Application and Evaluation of Entomopathogens for Control of Pest Insects in Mint. In *Field Manual of Techniques in Invertebrate Pathology: Application and Evaluation of Pathogens for Control of Insects and Other Invertebrate Pests*. 2nd ed. Edited by L.A. Lacey and H.K. Kaya, pp. 599-608. Dordrecht, The Netherlands: Springer.

Berry, R. 2000. Control of Root Weevils in Nursery Crops with Entomopathogenic Nematodes. In *Workshop Proceedings: Beneficial Nematode, Application in Greenhouse, Nursery and Small-Fruit Operations*, pp. 22–29. Edited by P. Gothro, Oregon State University.

Berry, R.E., J. Liu, and E. Groth. 1997. Efficacy and Persistence of *Heterorhabditis marelatus* (Rhabditida: Heterorhabditidae) Against Root Weevils (Coleoptera: Curculionidea) in Strawberry. *Environmental Entomology* 26(2): 465–470.

Campbell, J., and R. Gaugler. 1997. Inter-specific Variation in Entomopathogenic Nematode Foraging Strategy: Dichotomy or Variation Along a Continuum? *Fundamental & Applied Nematology* 20: 393–398.

Campbell, J., E. Lewis, F. Yoder, and R. Gaugler. 1996. Entomopathogenic Nematode Spatial Distribution in Turfgrass. *Parasitology* 113: 473–482.

Denno, R.F., D.S. Gruner, and I. Kaplan. 2008. Potential for Entomopathogenic Nematodes in Biological Control: A Meta-Analytical Synthesis and Insights from Trophic Cascade Theory. *Journal of Nematology* 40(2): 61-72.

Duncan, L. W., D. I. Shapiro, C. W. McCoy, and J. H. Graham. 1999. Entomopathogenic Nematodes as a Component of Citrus Root Weevil IPM. In *Workshop Proceedings: Optimal Use of Insecticidal Nematodes in Pest Management*, pp. 79–90. Edited by S. Polavarapu, Rutgers University.

Gaugler, R., J. Campbell, and T. McGuire. 1989. Selection for Host Finding in *Steinernema feltiae*. *Journal of Invertebrate Pathology* 54: 363–372.

Gaugler, R. 1999. Matching Nematode and Insect to Achieve Optimal Field Performance. In *Workshop Proceedings: Optimal Use of Insecticidal Nematodes in Pest Management*, pp. 9–14. Edited by S. Polavarapu, Rutgers University.

Gaugler, R., P. Grewal, H.K. Kaya, and D. Smith-Fiola. 2000. Quality Assessment of Commercially Produced Entomopathogenic Nematodes. *Biological Control* 17: 100–109.

Kaya, H.K., and R. Gaugler. 1993. Entomopathogenic Nematodes. *Annual Review of Entomology* 38: 181–206.

Kaya, H.K., and A.M. Koppenhöfer. 1999. Biology and Ecology of Insecticidal Nematodes. In *Workshop Proceedings: Optimal Use of Insecticidal Nematodes in Pest Management*, pp. 1–8. Edited by S. Polavarapu, Rutgers University.

Lewis, E.E. 1999. Handling, Transport, and Storage of Insecticidal Nematodes. In *Workshop Proceedings: Optimal Use of Insecticidal Nematodes in Pest Management*, pp. 25–30. Edited by S. Polavarapu, Rutgers University.

Poinar, G.O. 1990. Biology and Taxonomy of Steinernematidae and Heterorhabditidae. In: *Entomopathogenic Nematodes in Biological Control*, pp. 23–61. Edited by R. Gaugler and H.K. Kaya. Boca Raton, FL: CRC Press.

Polavarapu, S. 1999. Insecticidal Nematodes for Cranberry Pest Management. In *Workshop Proceedings: Optimal Use of Insecticidal Nematodes in Pest Management*, pp. 79–90. Edited by S. Polavarapu, Rutgers University.

Ramsay, C., R. Hines, and C. Foss. 2009. Private Applicator Pesticide Education Manual: A Guide to Safe Use and Handling. *Washington State University Extension Publication* EM020.

Shapiro-Ilan, D.I., and R. Gaugler. 2010. Nematodes: Rhabditida: Steinernematidae & Heterorhabditidae. In: *Biological Control: A Guide to Natural Enemies in North America*. Edited by A. Shelton, Cornell University. http://www.biocontrol.entomology.cornell.edu/pathogens/nematodes.html.

Shetlar, D.J. 1999. Application Methods in Different Cropping Systems. In *Workshop Proceedings: Optimal Use of Insecticidal Nematodes in Pest Management*, pp. 31–36. Edited by S. Polavarapu, Rutgers University.

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PNW544