USING BENEFICIAL 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 (e.g., plant parasitic) and beneficial (e.g., entomopathogenic) nematodes. Non-beneficial nematodes cause damage to crops and other types of plants. Beneficial nematodes cause damage to soilborne insect pests, yet are not harmful to humans, animals, plants, or earthworms, and can therefore be used as biological control organisms. Beneficial nematodes are also referred to as "entomopathogenic" (causing disease within an insect) or "insecticidal" (ability to kill insects) (Figure 1).

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, beneficial 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. Beneficial nematodes have been released extensively in the field with negligible effects on nontarget insects and are regarded as exceptionally safe to the environment.

The keys to success with beneficial nematodes are: (1) understanding their life cycles and functions; (2) matching the correct nematode species with the pest species; (3) applying them at the right time; (4) using them under



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

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appropriate environmental conditions (temperature, soil moisture, sunlight); and (5) applying them prior to or after other pesticide applications.

Beneficial nematodes are living organisms. They require careful handling in order to survive shipment and storage as well as appropriate environmental conditions in order to survive in the soil after application.

LIFE CYCLE OF BENEFICIAL NEMATODES

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

Life Cycle of Beneficial Nematodes

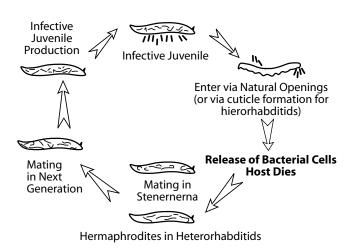


Figure 2. Diagram of the generalized life cycle of steinernematid nematodes (Grewal 1999a).

It is unknown whether introduced species of beneficial nematodes will overwinter in the region and successfully colonize insect hosts in subsequent years. Many species of beneficial nematodes occur naturally in the Pacific Northwest, including *Heterorhabditis marelatus*. *Heterorhabditis marelatus* has been shown to control some crop insect pests (Berry et al. 1997) and has just become commercially available.

HOW BENEFICIAL NEMATODES CONTROL INSECT PESTS

Only nematodes in the infective juvenile stage will survive in the soil and will find and penetrate insect pests. An understanding of host-finding strategies enables the user to properly match nematode species to pest insects to insure infection and control (Gaugler 1999). Infective juvenile beneficial nematodes locate their hosts in soil 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*; and *S. riobravis*, and *S. feltiae* do a bit of both ambushing and cruising (Campbell and Gaugler 1997).

Ambushing. 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 nematodes most effectively control insect pests that are highly mobile at the soil surface, such as cutworms, armyworms, and mole crickets.

Cruising. Nematodes that use the cruising strategy are highly mobile and are able to move throughout the soil profile. Cruisers locate their host by sensing carbon dioxide or other volatiles released by the host. Cruiser 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 nematodes will infect a single insect host. The infective juvenile nematodes penetrate the insect's body cavity, usually through natural body openings including the mouth, anus, or breathing pores (spiracles). Heterorhabditids can break the outer cuticle of the insect using a dorsal "tooth" or hook. Once inside the body cavity of the host, the infective juveniles release bacteria that live symbiotically within the nematode's gut. The nematode-bacterium relationship is highly specific: only *Xenorhabdus* spp. bacteria co-exist with steinermatids, 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. The insect larva is killed by the bacteria and not by the nematode. The bacteria do not harm the nematode.

The nematodes feed on both bacteria they release and host insect tissue. After a few days inside the host, nematodes mature to the adult stage (Figure 2). These adult nematodes produce hundreds of thousands of new juveniles. These progeny may undergo several life-cycles within a single host. 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 from 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). Duration of infective juvenile survival in soil is unknown. While in the soil they can become prey to invertebrates and microorganisms.

USING BENEFICIAL NEMATODES

Over 30 species of beneficial nematodes have been identified. Eight species have been commercialized worldwide and seven are currently available in the United States: *Steinernema carpocapsae*, *S. feltiae*, *S. glaseri*, *S. riobravis*, *Heterorhabditis bacteriophora*, *H. megidis*, and *H. marelatus*. The eighth species, *S.* *scapterisci*, although commercialized, is no longer available (Kaya and Koppenhöfer 1999).

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

Obtaining Beneficial Nematodes

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

Shelf Life

In general, beneficial 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 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 nematodes only 3–4 days prior to application. Nematodes should be shipped by overnight delivery in their infective juvenile stage and are most effective if used within 1–2 days after arrival. Examine the 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 and in 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. Examine a small sample from the shipment container, following instructions included with the shipment. In general, 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 60°F) 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 under a microscope. Live nematodes will be mobile and will have a bend to their shape. *S. carpocapsae* has a resting "J" shape and will move only when prodded with a pin or needle. All other nematodes will move in an "S" pattern (Lewis 1999). If the nematodes are straight and are not moving, it is likely they are dead. A mortality rate of 10% should be expected. If more than 20% of the nematodes are dead, inform your supplier immediately.

Nematodes should be stored in their shipment containers under refrigeration until ready for use. The storage life of nematodes is species- and formulationdependent. Specific storage instructions will be

TABLE 1. CURRENT USE OF STEINERNEMA AND HETERORHABDITIS NEMATODES AS BIOLOGICAL CONTROL ORGANISMS (Adapted from Gaugler 1999, and Polavarapu 1999).

CROP	INSECT PEST	NEMATODE SPECIES	
Artichokes	Artichoke plume moth	S. carpocapsae	
Berries	Root weevils	H. bacteriophora, S. glaseri, H. marelatus	
Cranberries	Root weevils Cranberry girdler Hoplia grub Cranberry rootworm	H. bacteriophora, S. carpocapsae S. carpocapsae, H. bacteriophora, H. marelatus H. bacteriophora H. bacteriophora	
Mint	Mint root borer, root weevils, mint flea beetle	S. carpocapsae	
Landscape ornamental trees and shrubs	Borers	S. carpocapsae	
Mushrooms	Sciarids	S. feltiae	
Ornamentals	Root weevils Wood borers Fungus gnats	H. bacteriophora, H. megidis, H. marelatus S. carpocapsae, H. bacteriophora S. feltiae	
Turf	Scarabs Billbugs Armyworm, cutworm, webworm, cranefly	H. bacteriophora H. bacteriophora, S. carpocapsae S. carpocapsae	
Vegetables— root and cole	Root maggots, cutworms, armyworms	S. carpocapsae	

TABLE 2. CHARACTERISTICS OF SEVEN SPECIES OF BENEFICIAL NEMATODES THAT ARE COMMERCIALLY AVAILABLE IN THE U.S. (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 70–85°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. Currently 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. Additional work needed for commercialization.
- *S. riobravis.* Combines ambusher and cruiser strategies, and attacks corn earworms, citrus root weevils, pink bollworms, and mole crickets. Isolated from 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. Currently has poor stability in formulation and a short shelf life. Additional work needed for successful commercialization.
- *H. marelatus.* 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. Newly available.
- *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 U.S. Currently has poor formulation stability and a short shelf life.

TABLE 3. REFERENCE LISTS OF COMMERCIAL SUPPLIERS OF BENEFICIAL NEMATODES.

Each Web site contains a list of retail suppliers of beneficial organisms including 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
- **Retail Suppliers of Beneficial Organisms.** 1994. Compiled by Robert Wright. Nebraska Cooperative Extension. http://www.ianr.unl.edu/pubs/insects/nf182.htm
- Suppliers of beneficial organisms in North America. 1997. State of California, Department of Pesticide Regulation, Environmental Monitoring, & Pest Management. Mailing address: 830 K Street Sacramento, CA 95814-3510 Phone: (916) 324 4100 http://www.cdpr.ca.gov/docs/ipminov/bscover.htm or /bensuppl.htm

included with the nematode shipment and should be followed. Table 4 is a summary of storage times for beneficial 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 storage life has expired, expect 70–100% mortality of the nematodes (Grewal 2000).

Soil Conditions

Beneficial 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 to successfully locate a host; at the same time they need 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. Soil temperatures between 77° and $82^{\circ}F$ are ideal for applying all nematode species. In general, soil temperatures greater than $85^{\circ}F$ can decrease the efficacy of some nematode species while soil temperatures less than $50^{\circ}F$ 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 $57^{\circ}F$ while *S. riobravis* can be effective at $95^{\circ}F$.

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. The soil can also be irrigated after nematodes have been applied. 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.

TABLE 4. EXPECTED STORABILITY OF SOME COMMERCIALLY AVAILABLE FORMULATIONSOF BENEFICIAL NEMATODES (adapted from Grewal 1999b).

		STORAGE (MONTHS)	
FORMULATION	NEMATODE SPECIES	ROOM TEMP.	REFRIGERATED
Liquid concentrate	S. carpocapsae	5–6 days	12–15 days
	S. riobravis	3–4 days	7–9 days
Sponge	S. carpocapsae	0	2–3
	H. bacteriophora	0	1–2
Vermiculite	S. feltiae	0	4–5
	H. megidis	0	2–3
Alginate gels	S. carpocapsae	3-4	6–9
	S. feltiae	¹ / ₂ -1	4–5
Flowable gels	S. carpocapsae	1-1 ¹ / ₂	3–5
	S. glaseri	0	1–1 ¹ /2
Water dispersible granules	S. carpocapsae	4–5	9–12
	S. feltiae	1 ¹ /2–2	5–7
	S. riobravis	2–3	4–5
Wettable powder	S. carpocapsae	2 ¹ / ₂ -3 ¹ / ₂	6–8
	S. feltiae	2-3	5–6
	H. megidis	2-3	4–5
Nematode wool	H. bacteriophora	21 days	unknown

Preparing for Application

Nematodes should be prepared for application no earlier than one hour prior to field application. 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. 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, highpressure, 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 (Ramsay and Thomasson 1995).

Remove all screens smaller than 50-mesh from the 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 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 will 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 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 beneficial 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 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 beneficial 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* (Ramsay and Thomasson 1995).

Pesticide Safety

Beneficial nematodes, like all biological control organisms, are considered a pesticide. Beneficial nematodes are exempt from federal pesticide registration under Section 25(b) of the Federal Insecticide Fungicide Rodenticide Act (FIFRA). However, all pesticides used or distributed must be registered with the State Department of Agriculture prior to distribution. Questions regarding pesticide registration should be directed to the State Department of Agriculture Pesticide Division.

- Washington State Department of Agriculture Pesticide Registration Section PO Box 42589, Olympia, WA 98504-2589 Phone: (360) 902-2030, Fax (360) 902-2093 Email: pestreg@agr.wa.gov http://www.wa.gov/agr/pmd/index2.htm
- Oregon State Department of Agriculture Pesticides Division 635 Capitol Street NE, Salem, OR 97301-2532

Phone: (503) 986-4635, Fax (503) 986-4735 Email: dblevins@oda.state.or.us http://pesticide.oda.state.or.us

• Idaho State Department of Agriculture Division of Agricultural Resources Pesticide Division PO Box 790, Boise, ID 83701 Phone: (208) 332-8593, Fax (208) 334-3547 Email: grobinso@agri.state.id.us http://www.agri.state.id.us/agresource agstandards.htm

Environmental benefits of using 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 the State Department of Agriculture Organic Program.

• Washington State Department of Agriculture Organic Food Program

PO Box 42560, Olympia, WA 98504-2560 Phone: (360) 902-1877, Fax (360) 902-2087 Email: organic@agr.wa.gov, http://www.wa.gov/agr/fsah/organic/index.htm

- Oregon State Department of Agriculture Food Safety Division 635 Capitol Street NE, Salem, OR 97031-2532 Phone: (503) 986-4720, Fax (503) 986-4729 Email: rmckay@oda.state.or.us http://www.oda.state.or.us/
- Idaho State Department of Agriculture Organic Program

PO Box 790, Boise, ID 83701 Phone: (208) 332-8673, Fax (208) 334-2170 Email: mmisner@agri.state.id.us http://www.agri.state.id.us

More Information About Beneficial Nematodes

Several internet sites provide detailed information about beneficial nematodes.

SARE Entomopathogenic Nematode Website

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 nematode authorities who will respond to questions.

The Gaugler Laborator—Rutgers University

http://www.rci.rutgers.edu/~nematode/ Emphasizes a balance of basic and applied research to achieve fundamental understandings of the insectnematode host-parasite relationship to increase efficacy and integration of entomopathogenic nematodes in pest management systems. Includes a slide collection, list of publications, and summaries of current projects including production, behavioral and developmental ecology, and genetic engineering of entomopathogenic nematodes.

Research on Entomopathogenic Nematodes— Oregon State University

http://www.bcc.orst.edu/~jiel/ or http:// www.bcc.orst.edu/epnematode An interdisciplinary team with expertise on biological control, nematode taxonomy, molecular systematics, population and quantitative genetics, quantitative ecology, and physiology. Includes abstracts of recent publications, images of insect pests infested with nematodes, images of morphological characters of nematodes, and a table of phylogenic relationships among nematodes.

Nematodes as Biological Control Agents of Insects—University of Nebraska

http://ianrwww.unl.edu/ianr/plntpath/nematode/ wormepns.htm

Entomopathogenic nematodes for the biological control of insects. Includes discussion of the species *Steinernema* and *Heterorhabditis* including culture, storage, transport, and images. Includes an electronic copy of the paper.

Midwest Biological Control News

http://www.entomology.wisc.edu/mbcn/mbcn.html Provides current and back issues of Midwest Biological Control Newsletters. Also includes an alphabetical index of biological control-related articles. For example, clicking on the N button will provide a list of topics including nabid bug, National Academy of Science Report, *Nealiotus curculionis*, and nematodes. Click on any of the topics for complete article listing.

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