UF UNIVERSITY of FLORIDA
IFAS Extension

EENY-530

Entomopathogenic Nematodes (Nematoda: Rhabditida: families Steinernematidae and Heterorhabditidae) [1]

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Introduction

Entomopathogenic nematodes are soft bodied, non-segmented roundworms that are obligate or sometimes facultative parasites of insects. Entomopathogenic nematodes occur naturally in soil environments and locate their host in response to carbon dioxide, vibration, and other chemical cues (Kaya and Gaugler 1993). Species in two families (Heterorhabditidae and Steinernematidae) have been effectively used as biological insecticides in pest management programs (Grewal et al. 2005). Entomopathogenic

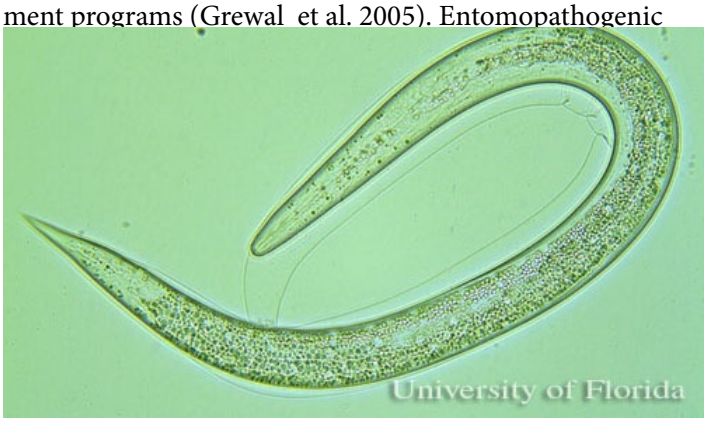

Figure 1. Infective juvenile stages of Steinernema carpocapsae clearly showing protective sheath formed by retaining the second stage cuticle. Credits: James Kerrigan, University of Florida.

nematodes fit nicely into integrated pest management or IPM programs because they are considered nontoxic to humans, relatively specific to their target pest(s), and can be applied with standard pesticide equipment (Shapiro-Ilan et al. 2006). Entomopathogenic nematodes have been exempted from the U.S. Environmental Protection Agency (EPA) pesticide registration.There is no need for personal protective equipment and re-entry restrictions. Insect resistance problems are unlikely.

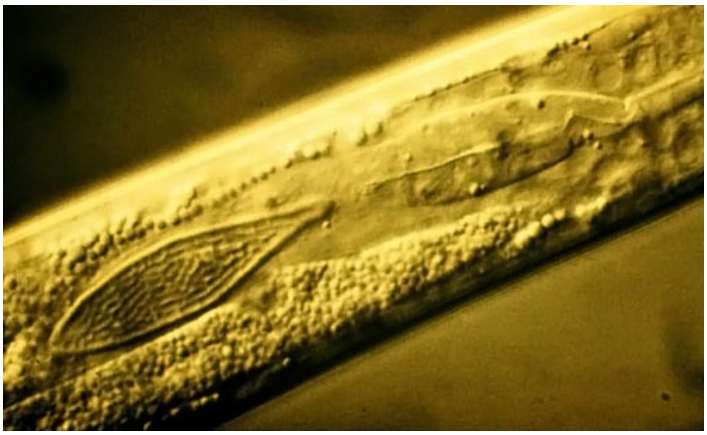

Figure 2. Symbiotic bacteria observed in intestinal tract of S. carpocapsae. Credits: Robin Bedding, CSIRO.

Life cycle

The infective juvenile stage (IJ) is the only free living stage of entomopathogenic nematodes. The juvenile stage penetrates the host insect via the spiracles, mouth, anus, or in some species through intersegmental membranes of the

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cuticle, and then enters into the hemocoel (Bedding and Molyneux 1982). Both *Heterorhabditis* and *Steinernema* are mutualistically associated with bacteria of the genera *Photorhabdus* and *Xenorhabdus*, respectively (Kaya and Gaugler 1993). The juvenile stage release cells of their symbiotic bacteria from their intestines into the hemocoel. The bacteria multiply in the insect hemolymph, and the infected host usually dies within 24 to 48 hours. After the death of the host, nematodes continue to feed on the host tissue. The nematodes develop through four juvenile stages to the adult, and then reproduce. Depending on the available resources, one or more generations may occur within the host cadaver, and a large number of juveniles are eventually released into environment to infect other hosts and continue their life cycle (Kaya and Gaugler 1993).

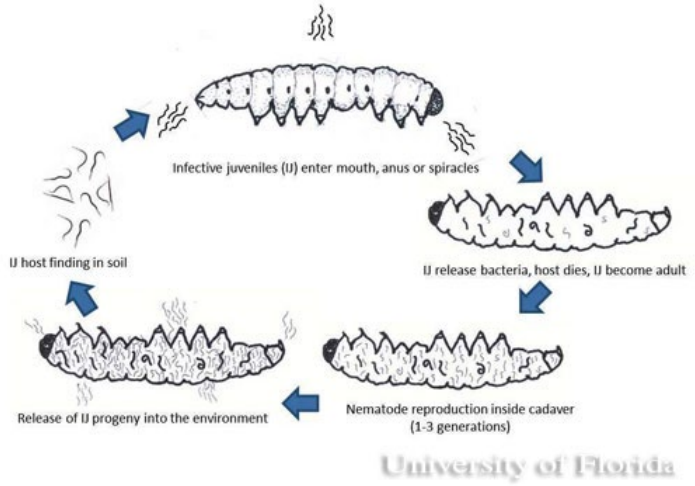

Figure 3. Generalized life cycle of entomopathogenic nematodes.
Credits: Steven Arthurs, University of Florida.

Reproduction differs in heterorhabditid and steinernematid nematodes. Infective juveniles of heterorhabditid nematodes become hermaphroditic adults, but individuals of the next generation produce both male and females whereas in steinernematid nematodes all generations are produced by males and females, (gonochorisism) (Grewal et al. 2005). The insect cadaver becomes red if the insects are killed by heterorhabditids and brown or tan if killed by steinernematids (Kaya and Gaugler 1993). The color of the host body is indicative of the pigments produced by the monoculture of mutualistic bacteria growing in the hosts.

Searching behavior

Entomopathogenic nematodes use two search strategies: ambushers or cruisers (Grewal et al. 1994a). Ambushers such as *S. carpocapsae* have an energy-conserving approach and lie in wait to attack mobile insects (nictitating) in the upper soil. Cruisers like *S. glaseri* and *H. bacteriophora* are highly active and generally subterranean, moving significant distances using volatile cues and other methods to find their host underground. Therefore, they are effective against less mobile pests such as white grubs (Scarab beetles). Some nematode species, such as *S. feltiae* and *S. riobrave,* use an intermediate foraging strategy (combination of ambush and cruiser type) to find their host.

Production and formulation

Entomopathogenic nematodes are currently produced by different methods either *in vivo* or *in vitro* (solid and liquid culture) (Shapiro-Ilan and Gaugler 2002). *In vivo* production is a simple process of culturing a specific entomopathogenic nematodes in live insect hosts, which requires

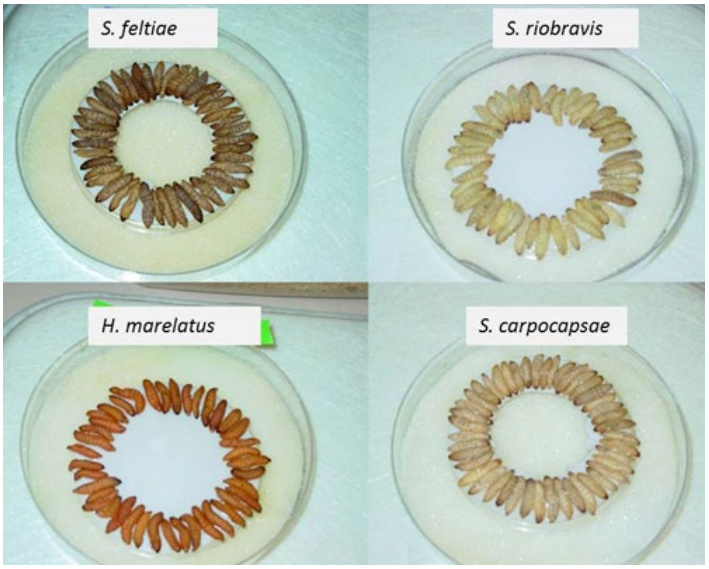

Figure 4. *In vivo* production of different entomopathogenic nematodes species in wax moth larvae using White traps; note the different colors of cadavers.
Credits: Heather Headrick, USDA.

minimal technology and involves the use of a surrogate host (typically larvae of wax moth (*Galleria mellonella*), trays and shelves. *In vivo* production uses a White trap (White 1927), which takes advantage of the juvenile stage's natural migration away from the host-cadaver. However, this method is not cost effective for scaled-up productions and may be only ideal for small markets (Shapiro-Ilan et al. 2002). *In vitro* culturing of entomopathogenic nematodes is based on introducing nematodes to a pure culture of their symbiont in a nutritive medium. Significant improvements in *in vitro* culture utilizing large fermenters are used by to produce large quantities of entomopathogenic nematodes for commercial use. Nematodes can be stored and formulated in different ways including the use of polyurethane sponge, water-dispersible granules, vermiculite, alginate gels and baits. Formulated entomopathogenic nematodes can be stored for 2 to 5 months depending on

Figure 5. Small scale *in vitro* of EPN in flask.
Credits: Lerry Lacey, USDA.

the nematode species and storage media and conditions. Unlike other microbial control agents (fungi, bacteria, and virus) entomopathogenic nematodes do not have a fully dormant resting stage, and they will use their limited energy during storage. The quality of the nematode product can be determined by nematode virulence and viability assays, age, and the ratio of viable to non-viable nematodes (Grewal et al. 2005).

Handling and effectiveness

Unsatisfactory results of entomopathogenic nematodes are caused by improper handling, transport, and storage (Shapiro-Ilan et al. 2002). Entomopathogenic nematodes are living organisms, and both biotic and abiotic factors affect entomopathogenic nematodes efficacy during applications. Entomopathogenic nematodes work best in sandy soil with a pH between 4 and 8. Entomopathogenic nematodes are susceptible to freezing, hot temperatures, desiccation, and UV light. *S. ribrave*, *S. glaseri,* and *H. indica* are among the more heat tolerant species, while *S. feltiae*, *H. megidis* and *H. marelatus* are adapted to cooler temperatures (Grewal et al. 1994b). The nematode efficacy can be enhanced by matching the most appropriate entomopathogenic nematodes species to the target pests, using the correct rate of a viable nematode product, keeping the treated area wet for at least 8 hours post application and applying during early morning or evening hours to minimize UV exposure and drying conditions. It is also important to inspect entomopathogenic nematodes after receiving them and prior to application to ensure that they are viable (sinusoidal movement of healthy juvenile stages can be observed with a 20 X hand lens or microscope).

Application considerations

Entomopathogenic nematodes can be applied with most horticultural equipment, including pressurized sprayers, mist blowers, and electrostatic sprayers. The application equipment chosen will depend on the cropping system. In general large diameter nozzles (orifices) and high volumes (> 100 gpa) are recommended. Filters, screens, and swirl plates should be removed from spray equipment lines to prevent them from becoming clogged with juvenile stages. It is also important to ensure adequate agitation during application because entomopathogenic nematodes settle quickly in suspension. High pressures > 300 psi should also be avoided and entomopathogenic nematodes can be kept cool by adding ice packs to the spray suspension. Studies have shown that entomopathogenic nematodes are compatible with many (but not all) insecticides, fungicides, and herbicides. Fresh manure or high rates of chemical fertilizers (e.g., urea) can be detrimental to entomopathogenic nematode's persistence and efficacy. Substantial progress has been made in recent years in developing entomopathogenic nematode formulations, particularly for aboveground applications, e.g., mixing entomopathogenic nematodes with particular surfactants and water dispersable polymers (Shapiro-Ilan et al. 2010).

Figure 6. Applying entomopathogenic nematodes in an orchard using tractor-mounted airblast sprayer configured for this purpose.
Credits: Lerry Lacey, USDA.

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Table 1.

EPN species	**Major pest(s) targeted- as recommended by various commercial companies**
S. glaseri	White grubs (scarabs, especially Japanese beetle, *Popillia* sp.)
S. kraussei	Black vine weevil, *Otiorhynchus sulcatus*
S. carpocapsae	Turfgrass pests- billbugs, cutworms, armyworms, sod webworms, chinch bugs. Orchard, ornamental and vegetable pests- codling moth, cranberry girdler, dogwood borer and other clearwing borer species, black vine weevil, peachtree borer, shore flies (*Scatella* spp.)
S. feltiae	Fungus gnats (*Bradysia* spp.), shore flies, western flower thrips
S. scapterisci	Mole crickets (*Scapteriscus* spp.)
S. riobrave	Citrus root weevils (*Diaprepes* spp.)
H. bacteriophora	White grubs (scarabs), cutworms, black vine weevil, flea beetles, corn root worm
H. megidis	Weevils
H. indica	Fungus gnats, root mealybug, grubs
H. marelatus	White grubs (scarabs), cutworms, black vine weevil