Rearing Entomopathogenic Nematodes with Household Items
Joseph Tourtois and Matthew Grieshop
MSU Organic Pest Management Laboratory

Entomopathogenic nematodes are small round worms that naturally occur in the soil. There are two families of entomopathogenic nematodes: Heterorhabditidae and Steinernematidae. They are obligate parasites of insects and they vector a symbiotic bacteria, hence the name entomopathogenic. The nematode-bacteria complex is highly virulent killing insect host with in 24-48 h. Because of their high virulence, they have been used as biopesticides for several decades. In the 1930's, they were first used to manage Japanese beetles. Today, farmers use entomopathogenic nematodes to manage several pests in various cropping systems including western flower thrips and fungus gnats in greenhouses, black vine weevil in nurseries, white grubs in turfgrass, cutworms in gardens, and codling moth in orchards.

The life cycle of entomopathogenic nematodes consists of: egg, four juvenile stages, and an adult stage (Fig. 1). Immature stages of nematodes are referred to as juveniles so as not to be confused with the immature stage of insects known as larvae (Fig. 2). Most of the nematode life cycle occurs within a host. The third stage infective juvenile (or dauer larva) is the only stage found outside of a host and does not feed. This stage vectors the symbiotic bacteria by carrying it in their intestine and infecting new hosts. To protect itself in the environment, the infective juvenile continues to wear the cuticle of the second stage as an extra sheath. Infective juveniles enter insect hosts through natural openings – mouth, anus, and spiracles.

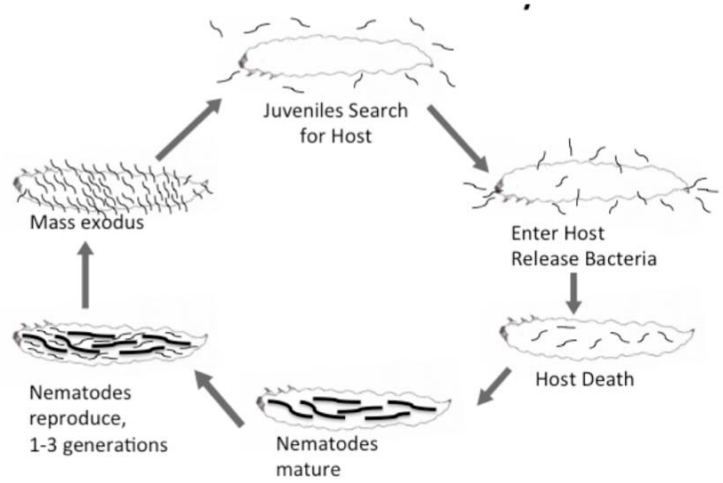

Figure 1. Entomopathogenic nematode life cycle. See text for description.

On the way into the host, the infective juvenile sheds the sheath. Once inside the insect haemocoel, the infective juvenile release the symbiotic bacteria. Together the nematode-bacteria complex overcome the host's immune system and kills it within 24-48 h. Then they proceed to release enzymes that breakdown the host into a nutrient soup. As the bacteria digest the host, they multiply. Nematodes feed on the bacteria and the nutrient soup of the digested host. They complete their development to adults within two to four days. The nematodes proceed through one to three generations dependent upon the size of the host. When host resources are depleted and the nematodes reach a certain density, juveniles develop into infective juveniles, store symbiotic bacteria in their intestinal cavity, and emerge from the cadaver by the thousands in search of a new host. Up to 200,000 nematodes can be reared in a single host.

Commercially reared nematodes are available for purchase through biological control companies. However, certain formulations to reduce shipping costs are not

approved for organic agriculture. Organically approved entomopathogenic nematodes are very limited and cost $200-$300/acre/application. Infective juveniles are applied to the soil or foliar canopy with spray equipment. The filters need to be removed. Nematodes are applied at rates of 1 billion nematodes per acre (2.5 billion per hectarce). Nematode need to be massed reared to apply at these high rates. Farmers can easily rear their own entomopathogenic nematodes *in vivo* with household items and wax worms.

Rearing nematodes requires a simple system of trays and shelves. Materials include: 9 inch aluminum pie plates with lids; 6 inch Styrofoam or plastic plates; coffee filters or paper towel; rubber bands or paper clips; shallow Tupperware containers; a 10x magnifying lens; eye dropper, syringe, or other pipette to transfer nematodes; water; and a warm, dark space. A rearing host is also need. Wax worms and mealworms are the two most commonly used rearing host.

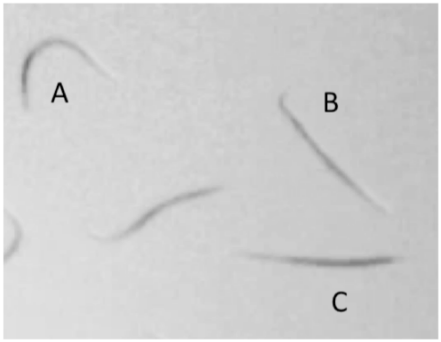

Figure 2. Infective juveniles. A. a healthy wriggling nematode. B. a healthy resting nematode. Note head is turned to give j-shape or hooked appearance. C. a dead nematode, its body is straight.

A nematode colony can be started from nematodes purchased from biological control companies. Wax worms and mealworms are typically available at local bait and pet stores. There are four main steps to the nematode rearing process: 1. assess nematode viability. 2. host infection, 3. transfer infected hosts, 4. harvest and storage.

1. Assess nematode viability. Most companies ship nematodes in a gel or sponge. Follow their directions to release the nematodes into water. Underneath 10x magnification assess the viability of infective juveniles in aqueous solution. Healthy nematodes are actively wiggling or in a resting J-shape with their head held at a slight angle to the rest of their body. Dead nematodes are perfectly straight. If more than 50% of the nematodes are dead, seek out a better source.


Figure 3. Infection chamber. Infective juveniles (20 – 100 per host) in water are applied to a paper towel in aluminum pie plate. Wax worms (5-25) are placed on the wet paper towel. The infection chamber is then covered with lid and placed in the dark.

2. Host infection. Wax worms need to infected with 20-25 infective juveniles. Too few nematodes and we you may experience a poor infection rate. Too many nemtodes and you will experience reduced production due to competition. Yellow mealworms need to be infected with 400-800 infective juveniles. Prepare infection chamber by arranging 5 to 25 wax worms (i.e. the host) on the paper towel inside of pie plate (Fig. 3). Using a pipette or similar

tool, apply 20-100 infective juveniles (IJ) per host in water. The paper should absorb all of the water. Nematodes move through the water film surrounding the paper fibers. Too little to too much water will hinder nematode movement. Cover the pie plate with lid and secure in place with rubber band or paper clips. Place pie plates in a humid, dark space (e.g. under a cardboard box) at room temperature (70 - 75 °F). Nematodes will crawl around on the wet paper to search for the wax worms.

3. Transfer infected hosts to harvesting chamber. Four to seven days later check insect mortality. The wax worms should be dead and changing color. *Heterorhabditis spp.* will turn the host red colors and glow in the dark. *Steinernema spp.* will turn the host gray or beige colors. Remove any hosts that are still alive or that have turned black or smell putrid. Black wax worms are infected with some other pathogen, not nematode infected (Fig. 4).

Figure 4. Infected wax worms. A. wax worms infected with *Heterorhabditis spp.* turn red colors. B. wax worms infected with *Steinernema spp.* turn gray or beige colors.

To prepare the harvesting chamber, place the paper towel with infected wax worms onto a 6-inch Styrofoam plate. Fold the edges of the paper towel underneath itself so that they do not extend beyond the edge of the plate. Remoisten the paper towel to aid nematode movement. Pour approx. 1 cup of water into the pie plate and float the Styrofoam plate on the water. Within the next week, nematodes will emerge from the cadavers, traverse the moist paper, and settle into the water. Reattach the lid and place in a dark space.

4. Harvest and storage. Check the plates daily for nematodes in the water. Nematodes can be harvested once the water has become cloudy with thousands of nematodes. Harvest the nematodes by pouring the cloudy water from the pie plate into a shallow Tupperware container (Fig. 5). Wash the nematodes by adding extra water to the container and gently mixing the water by swirling the container. Allow nematodes to set to the bottom of the container for 10 minutes, and then pour off of the excess water. Repeat washing 1-2 times to remove foreign material. After the final pour-off of excess water, the water depth should not exceed ¼ inch for storage. Puncture holes in the lid with a nail to allow air exchange and cover the container. Nematodes require oxygen to live. They will die in anaerobic conditions if too many nematodes are present in the water or the water is too deep. There are too many nematodes in the water if you cannot see through the water. Store the nematodes at room temperature in a dark place. Nematodes are very

Figure 5. Nematodes ready for harvest.

sensitive to ultraviolet light. Two minutes of exposure to ultraviolet light will kill them. If stored properly, they can survive in water for about a month.

The total time to rear entomopathogenic nematodes is 2-4 weeks depending on species and temperature. *Heterorhabditis spp.* usually develops slower than *Steinernema spp.* Since the rearing process takes a several weeks, it is advisable to continually rear entomopathogenic nematodes.

When rearing multiple species, be careful not to contaminate the species. A couple of ways to avoid contamination is to label species with different colors and handling only one species per day. If two nematode species enter one host, they will compete in the host. Only one species will reproduce and emerge from the infected host. Determining which species successfully reproduced is difficult. It requires slide mounting and expert knowledge.

Apply nematodes in aqueous solution as a soil drench using standard spray or irrigation equipment. Select nozzles with an aperture that is greater than 0.5 mm; and do not exceed 300 psi. Evening is the best time to apply nematodes since they are sensitive to sunlight. Apply extra water to wash nematodes down into the soil. Alternatively, the infected cadavers can also be placed on the soil surface to allow the nematodes to emerge directly into the soil.

Further Resources:

Barbercheck M (2014) Insect-Parasitic Nematodes for the Management of Soil-Dwelling Insects. In: Entomology (Penn State University). http://ento.psu.edu/extension/factsheets/parasitic-nematodes. Accessed 26 Jun 2014

Dindia J (2014) Rearing of Entomopathogenic Nematodes. Michigan State University Organic Pest Management Laboratory, East Lansing, MI

Himmelein J, Grieshop M (2012) Regenerative Biological Control of Greenhouse Pests with Steinernema feltiae Nematodes.

Shapiro-Ilan DI, Randy Gaugler Nematodes (Rhabditida: Steinernematidae & Heterorhabditidae). In: Biological Control. http://www.biocontrol.entomology.cornell.edu/pathogens/nematodes.php. Accessed 24 Jan 2015

Acknowlegements:

This bulletin was produced using funds from the CERES trust. www.cerestrust.org