Biological Control of Insects

Thursday morning 9:00 am

Where: Grand Gallery (main level) Room C

This workshop will provide information on what biological control agents of insect pests look like, what they feed on, and how they can be managed on farm to increase their efficacy. You will get to see and handle pinned specimens, so you can learn to identify them.

MI Recertification credits: 3 (COMM CORE, PRIV CORE)
OH Recertification credits: 2 (presentations as marked)
CCA Credits: PM(3.0)

Moderator: Ben Phillips, Vegetable Educator, MSU Extension, Saginaw, MI

9:00 am	Introduction to Biological Control (OH: CORE, 0.5 hr)
	• Ben Phillips, Vegetable Educator, MSU Extension, Saginaw, MI
9:30 am	Predator, Parasitoid, and Prey Relationships (OH: CORE, 0.5 hr)
	• Adam Ingrao, Entomology Dept., MSU
10:00 am	Rearing Nematodes for Agriculture
	• Joe Tourtois, Entomology Dept., MSU
10:30 am	Habitat Management for Beneficial Insects
	• Nicole Quinn, Entomology Dept., MSU
11:00 am	Natural Enemies Identification (OH: 2B, 1.0 hr)
	• Ben Phillips, Vegetable Educator, MSU Extension, Saginaw, MI
12:00 noon	Session Ends

Rearing Nematodes for Agriculture

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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 used entomopathogenic nematodes to manage several pests in various cropping systems including western flower thrips and fungus gnats in greenhouses, fungus gnats in mushrooms, black vine weevil in nurseries, white grubs in turfgrass, and the peach tree borer and lesser peach tree borer in orchards.

The life cycle of entomopathogenic nematodes consists of: egg, four juvenile stages, and an adult stage. Immature stages of nematodes are referred to as juveniles so as not to be confused with the immature stage of insects known as larvae. Most of the nematode life cycle occurs within a host. The third stage infective juvenile (or dauer juvenile) 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 (Kaya and Gaugler 1993).

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 kill it within 24-48 h (Kaya and Gaugler 1993). 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 adult within two to four days. The nematodes proceed through one to three generations dependent upon the size of the host (Kaya and Gaugler 1993). 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 (Kaya and Gaugler 1993).

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. Farmers can easily rear their own entomopathogenic nematodes with household items and greater wax moth larvae (a.k.a. wax worms). Up to 200,000 nematodes can be reared in a single wax worm.

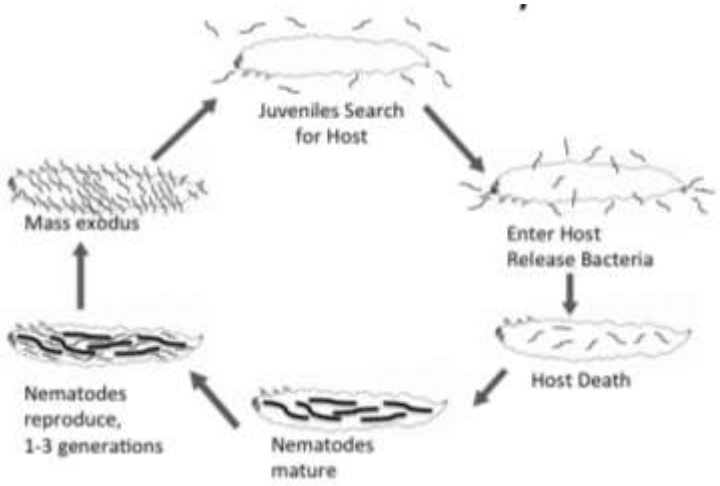

Figure 1. Entomopathogenic nematode life cycle. See text for description.

To start a nematode colony, farmers can initially purchase them from biological control companies. They will also want to purchase wax worms, commonly available at bait-and-tackle and pet stores. Rearing nematodes requires a simple system of trays and shelves (Shapiro-Ilan et al. 2014). Materials include: aluminum pie plates with lids; small Styrofoam plates; napkin or paper towel; rubber band; pipette, eye dropper, or syringe; shallow storage container; warm, dark space; and water. Since the nematodes are quite small, a dissection microscope capable of 20x magnification is quite beneficial for counting nematodes and becoming familiar with them.

Prepare infection chamber by arranging 5 to 25 wax worms (i.e. the host) on the paper towel inside of pie plate. Using a pipette or similar tool, apply 20-100 infective juveniles (IJ) per host in aqueous solution (Kaya and Stock 1997). The paper should absorb all of the water. Too much water will hinder nematode movement. Cover the pie plate with lid and secure in place with rubber band. Place pie plates in a moist, dark space (e.g. under a cardboard box) at room temperature. Nematodes will crawl around on the wet paper to search for the wax worms. The optimal rearing temperature for most entomopathogenic nematodes is 70 - 75 °F.

Two 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. Black wax worms are not nematode infected.

Tens days after set-up, it is time to prepare the harvesting chamber. Place a small Styrofoam plate under the napkin. Fold the edges of the napkin under so that they do not extend beyond the edge of the plate. Moisten the paper to aid nematode movement. Pour approx. 1 cup of water into the pie plate to float the Styrofoam plate. The nematodes will emerge from the cadavers within the next week, traverse the moist paper, and settle into the water. Harvest the nematodes by pouring them into a shallow container. Water depth should not exceed ½". Store the nematodes at room temperature in a dark place. Nematodes are very sensitive to ultraviolet light. Two minutes of exposure to ultraviolet light will kill them. Nematodes require oxygen to live. They will die in anaerobic conditions if too many nematodes are present or the water is too deep. 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 long time, it is advisable to continually rear entomopathogenic nematodes.
When rearing multiple species, be careful not to contaminate the species. If two nematode species enter one host, they will compete and only one species will reproduce. Determining which species successfully reproduced is difficult and requires slide mounting.

Apply nematodes 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 Reading:

Himmelein J, Grieshop M (2012) Regenerative Biological Control of Greenhouse Pests with *Steinernema feltiae* Nematodes. Michigan State University Extension

Kaya HK, Gaugler R (1993) Entomopathogenic Nematodes. Annual Review of Entomology 38:181–206. doi: 10.1146/annurev.en.38.010193.001145

Kaya HK, Stock SP (1997) Techniques in insect nematology, in: Manual of Techniques in Insect Pathology, L. A. Lacey ed. Academic Press, London, pp. 281–324

Shapiro-Ilan DI, Han R, Qiu X (2014) Chapter 10 - Production of Entomopathogenic Nematodes. In: Morales-Ramos JA, Rojas MG, Shapiro-Ilan DI (eds) Mass Production of Beneficial Organisms. Academic Press, San Diego, pp 321–355