Regenerative Biological Control of Greenhouse Pests with *Steinernema feltia* Nematodes

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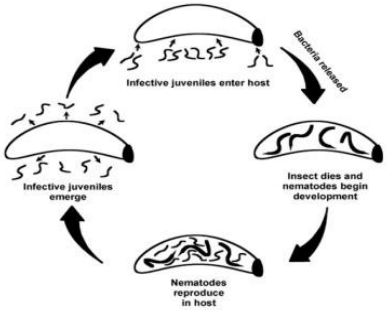
Biological Control with *Steinernema feltiae* nematodes?

The use of *S. feltiae* —an entomopathogenic nematode or EPN— for the control of fungus gnats and shore flies is gaining popularity. However the cost of purchasing EPN is prohibitive for many greenhouses. In a greenhouse propagation system, the environment is conducive to a high population of fungus gnats due to the moisture and temperature requirements. *Steinernema feltiae* is most effective at infecting fungus gnat larvae in the moist media where the fungus gnats populate. Regenerative biological control using *S. feltiae* involves their on-site rearing using *Galleria mellonella* larvae (wax worms). Wax worms are an ideal host and yield up to 100,000 infected juveniles per host larvae.

How it works?

Nematodes are aquatic or soil dwelling simple roundworms that are colorless, unsegimented and lack appendages. They maybe free-living, predaceous or parasitic. Many of the parasitic species cause unwanted diseases of plants, animals, and humans. Enomopathogenic nematodes —incuding *S. feltiae*— have a symbiotic relationship with bacteria (*Xenorhabdus* spp.) that are highly toxic to insects. The bacteria are released from the nematode's gut after it enters a host insect through the anus, mouth or spiracles. The bacterium rapidly multiplies causing rapid death within 24-48 hrs. Juvenile nematodes become adults and feed on the bacteruim and new IJ emerge from the host. Many thousands of IJ emerge from each infected host larva making EPN relatively simple to rear in large numbers.



Protocol to rear S. feltiae

Entomopathogenic nematodes reproduce quickly and progress from initial host infection to emergence of IJ in approximately 10 to 15 days at 65-75° F. Infecive juveniles are collected from the host larvae using a "white trap" or "float" system. The "float" system consists of two nested petri dishes with an infected host placed on moistened double layered filter paper on the small dish which in turnn is "floated" on water in the larger Petri dish. This allows the IJ to migrate from the host to the free water where they can be easily collected. Collected IJs can then be stored in aerated water.

Photo Credit: Jeanne Himmelein

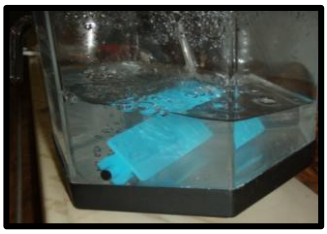


Photo Credit: Jeanne Himmelein

Supplies needed:

- Petri dishes, 2 sizes one smaller and one larger for floating, 150 mm and 100 mm, filter paper 90mm
- *Galleria mellonella* larvae (wax worms)
- *S. feltiae* infected juveniles' to infect wax worms
- Dissecting microscope or 20X or stronger hand lens
- Aerated storage container for nematodes (small fish tank with sufficient low pressure aeration).

- Drinking water or spring water (not distilled)
- Pipettes and tweezers to handle wax worms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Steps:

- Inspect waxworms and remove any that appear unhealthy. Healthy waxworms should be vigorous and lack any dark coloration near the head or hind end. Gently rinse wax worms prior to use with water.
- Nematodes are very sensitive to light. Infections and colonies should be kept in a darkened room, drawer, or under a box.
- Place 15 to 20 wax worms into a perti dish with a double layer of filter paper. Infect the wax worms by adding IJ from an existing colony. Add an abundant amount of the IJ from the water solution to saturate all the layers of filter paper without having to much excess that could be poured out of the dish. Place the top over the dish. Make air vent holes in the lid by heating a safety pin and melting small holes all over the lid. Mark the lid with the date of the infection. Re-check after a half hour to determine moisture level. Re-check at 24 and 48 hrs to determine if more IJ needs to be added for complete infection. Determine infection status at 48 hours —the wax worms should be immobile and their color should have change to a light tan almost bronze. If they become black or very rigid, remove them from the dish and discard them. (Figure 4)


Figure 4
Photo Credit Jeanne Himmelein

- Transfer all the infected wax worms onto a new dry double filter paper in a new dish. Cover with NON-vented top and keep them dry for 5-7 days.
- On the 7th to 10th day, add moisture to the filter paper and "float" the dish uncovered into the large petri dish filled with drinking water. To manage the moisture level on the wax worms, you may cover the large petri dish with its top but make sure the floating dish doesn't touch the lid. (Figure 5)
- Monitor the water daily for nematode activity and collect by pouring water into the aerated container. Replace the "float" water daily. The nematodes need fresh or aerated water for survival. The storage container should be placed in a cool location between 40-50°F.
- As the IJ emerge from the host wax worm, the wax worm will become smaller and shrivel up almost disintegrated. Continue to collect the float water until very few nematodes emerge and then discard or place host wax worms directly onto moist media to allow any remaining IJ to enter the media.


Figure 5
Photo Credit: Jeanne Himmelein

Trouble Shooting:

Poor infection

- Make sure the wax worms are healthy: they should be active and a creamy color without dark discoloration.
- Make sure to apply IJ that are alive and at a high rate to insure entrance into the wax worms. Dead IJ are completely straight and do not move when prodded with a pin.

Petri dishes smell rotten

- The samples are getting infected with bacteria. Increase air by keeping the petri dishes uncovered. This occurs more often if infected wax worms are not transferred to a clean dish with new filter paper. They will exude bodily fluids or feces prior to death opening up the potential for bacteria due to the high moisture levels.

Low emergence of JI from host

- Make sure there is enough moisture on the filter paper to allow them to move out into the water but not too wet providing and environment they will not want to leave and move out to the water for collection.
- The air temperature is either to hot or cold. The optimum range is in the 60's and 70's.