Entomopathogenic Nematodes for Control of the Asiatic Garden Beetle in Field Crops

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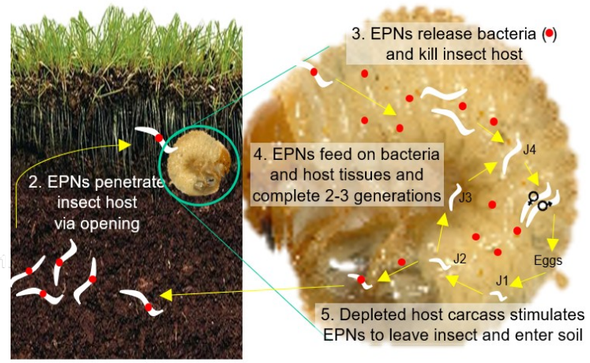
This handout is designed to educate readers how certain naturally occurring nematodes kill insect pests, and how they can be isolated from the field, mass-reared, and applied back into the field for potential long-term control of Asiatic garden beetle grubs in field crops. Additionally, benefits and disadvantages to using locally isolated nematodes for grub control are discussed.

What are entomopathogenic nematodes (EPNs)?

entomopathogenic* = *insect killing

Unlike plant parasitic nematodes, entomopathogenic nematodes only cause disease to insects, NOT plants.
EPNs are parasites only of soil dwelling insects and they belong to the nematode families Steinernematidae and Heterorhabditidae. They are present in all soil types and cause disease and rapid death to an insect host via blood poisoning. EPNs are compatible with insecticides and resistant varieties and may even act synergistically. Additionally, they have minimal off-target effects on applicators.

Typical EPN life cycle



What is Asiatic garden beetle?

Introduced to the U.S. from east Asia in 1921, Asiatic garden beetle (AGB) (A), an annual white grub species, spread to 24 states and 2 Canadian provinces as of 2012. AGB feeds on 100+ plant species and is a frequent pest of turf, ornamentals and vegetables. In 2007 in northern Indiana and southern Michigan, and 2013 in northwest Ohio, AGB grubs emerged as early-season root feeding (B) pests of field corn which cause seedlings to wilt and discolor (C), stunt (D), and ultimately die resulting in extensive stand losses. Infestations are often observed in sandy soils (E-F). Many insecticides are seemingly ineffective against AGB, however, EPNs are commonly used for management in turf.

Applying EPNs in the field

Things to consider:

Apply EPNs when it is either cloudy or around sunset to avoid UV rays which can kill EPNs.

EPNs require non-chlorinated water to move in the soil; chlorine will kill EPNs.

Agitate EPNs in tank during application to ensure a constant rate as they settle to the bottom.

Apply anytime during the growing season before September 1; ideally when soil temperatures are between 50-80°F and soils have moisture.

Application rates: Sprayers should be calibrated at 20-25 gallons per acre and EPNs applied at a rate of 100-300 million per acre.

Sprayer requirements

Remove screens and filters, and use fertilizer steam nozzles (006-0015) in order to prevent the EPNs from clogging.

Open nozzle bodies and let EPNs dribble out in a stream of water.

Why use naturally occurring EPNs instead of commercially available EPNs?

Commercially available EPN strains are readily obtainable and useful in controlled settings, however, there are many problems when using them for long-term biological control in field cropping systems. In general, commercial EPNs are adapted to laboratory conditions and struggle to infect hosts and persist in field settings in the absence of hosts, especially in extreme weather events, and they are relatively expensive to use when applied annually at high rates. Several benefits of using local EPNs are listed below.

Native EPNs are generally applied only once and at a fraction of the rate required for commercial EPNs	Persist for several years after application, and can live several months in the absence of insect hosts.	Locally adapted to environmental extremes instead of laboratory conditions.	High fecundity allows a single EPN to produce hundreds of thousands of offspring inside a single insect host.

Additional resources

- Please refer any questions or comments to Adrian Pekarcik (pekarcik.4@osu.edu).
- Additional information about AGB can be found at *www.aginsects.osu.edu*
- Dr. Elson Shields' lab at Cornell University has done extensive work troubleshooting and evaluating the use of naturally occurring EPNs for successful biological control of the Alfalfa snout weevil in New York alfalfa. More information regarding their work can be found at: *http://www.alfalfasnoutbeetle.org/index.php/resources-contacts*

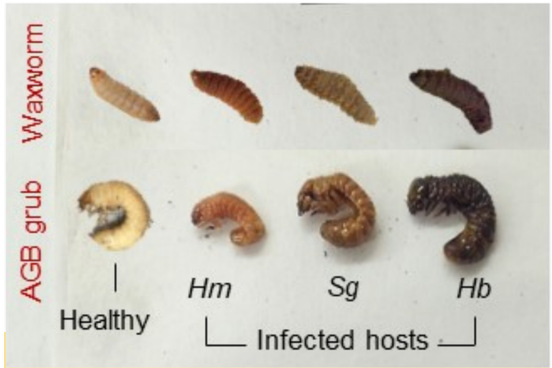
Cornell University

Isolation, identification, and extraction of EPNs for mass production

1. Field isolation

EPNs can be isolated from any agricultural field by taking soil samples from several areas and adding waxworms, which are naturally susceptible to nearly all EPNs, for bait. EPNs present in the soil will infect the waxworms and cause them to die and discolor. AGB grubs discolor similarly when infected.

If waxworms remain healthy after 3 days in the soil, then no EPNs were isolated and the process should be repeated with new soil samples until waxworms become infected.

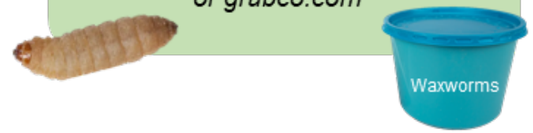


Within 48 hours of infection grubs and waxworms turn a distinct color depending on the EPN species. In general, both grubs and waxworms infected with *H. bacteriophora* (Hb) turn dark brick red, *H. megidis* (Hm) orange, and *S. glaseri* (Sg) tan.

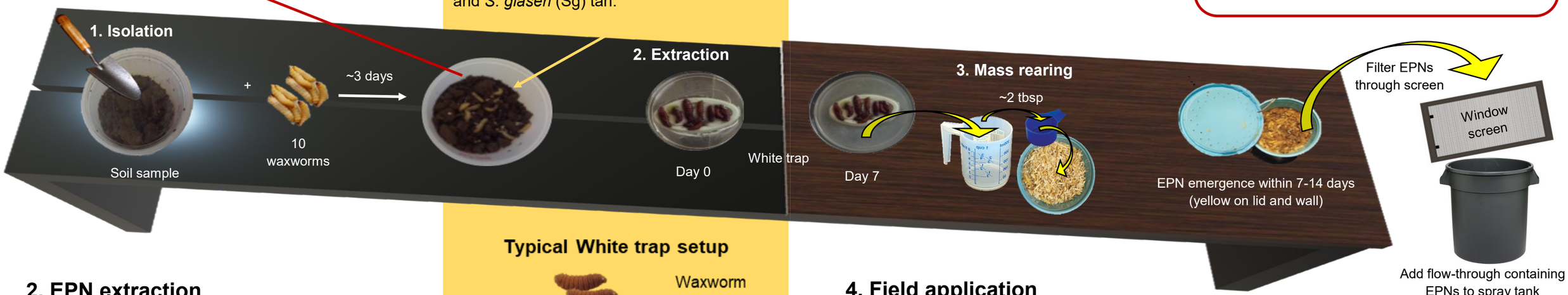
3. Mass production

Waxworms are used to mass produce EPNs that emerged into the White trap water. Approximately 2 tablespoons of water containing EPNs is poured over 250 waxworms in a container and maintained at room temp in the dark with constant moisture. EPNs emerge from infected waxworms within 2 weeks; aggregates of EPNs (which look yellow) appear on the container lid and walls. EPNs are separated from the waxworms and bedding into a large container using a sieve and distilled water. EPNs in the water solution are ready for field application.

Purchase waxworms from your local bait shop, or online vendors like rainbowmealworms.net, bestbait.com, speedyworm.com, or grubco.com



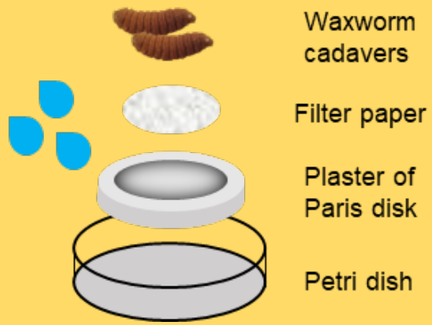
Emerged EPNs are collected by washing them from the waxworms and substrate through a window screen into a container like a garbage can. The flow through contains EPNs and will be added to the spray tank.



2. EPN extraction

EPNs are extracted from the insect host using a ***White trap (right)*** which moves EPNs from the host into water; EPNs require moisture to move and survive. The White trap works by placing infected waxworms onto a filter paper which is on top of a plaster of Paris disk that is surrounded by water. EPNs are attracted to water and move to it after emerging from the insect host. EPN emergence is detected when the water turns cloudy, or about 7-10 days after setup.

Typical White trap setup



The overall goal of the White trap is to elevate insect cadavers above water while ensuring there is moisture for EPNs to move into water after emergence.

4. Field application

Once the total amount of EPNs needed for field application is determined and enough of them have been mass produced and sieved, they can be applied with modified spray equipment (see back page). EPNs are sieved from waxworm containers by pouring the contents onto a screen and rinsing them with non-chlorinated water. The resulting water-EPN solution is added to the tank and applied around sunset (to avoid UV desiccation) on moist soils. EPNs are readily dispersed throughout the field when soil is moved (i.e., tilling).

Total EPNs per waxworm cup

Total waxworm cups per acre