Cornell CALS

College of Agriculture and Life Sciences

Entomology

Biological Control with Persistent Entomopathogenic Nematodes: Rearing, Application, Bioassays, and Persistence Preservation Protocols 2019

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Entomopathogenic Nematodes (EPNs)

(Insect-Attacking Nematodes)

Background

- As a group, entomopathogenic nematodes (EPN) have a broad host range, variation in foraging strategies, and host associations
- EPNs have the potential to control many insect pest species with diverse life histories as long as at least one life stage is passed beneath the soil surface
- Naturally occurring or adapted EPNs have shown to be effective in long-term suppression of pest outbreaks in managed systems, ranging from turf to agricultural fields
- EPNs provided by the Shields' Lab, Cornell University are native NY populations that have been isolated from numerous samples of NY agricultural soil samples

Background

- EPNs origins and future considerations should primarily be focused as an inoculative and conservation biological control

Biological Control

- Biological control is accomplished by inoculating fields with Entomopathogenic nematodes (EPNs)

- Cornell University, in collaboration with NY Farm Viability Institute and Northern NY Agricultural Development Program, developed a low-cost bio-control protocol to reduce current infestation levels and to help prevent further spread of insects

- Biological control using EPNs is currently the only effective and readily available method to combat alfalfa snout beetle and root weevil complex in Northern NY

- Close soil contact makes pests susceptible to attack by EPNs which are insect attacking nematodes

- The nematode life stage released from host insects is called an infective juvenile (IJ)

- Long-term biological control appears to be a feasible management strategy and studies indicate that EPNs can persist in soils for a number of years

  (Shields, E.J., A.M. Testa, and W.J. O'Neil. 2018. Long-term Persistence of Native NY Entomopathogenic Nematode Isolates across Crop Rotation. Journal of Economic Entomology 111(6), 2592-2598.)

- Soil insects are exposed to EPNs at different stages of their life cycle and at various depths within the soil profile

- Because different EPN species have different preferred soil profile niches that vary by depth, the multi-species approach mixes EPN species to cover the root zone of the plant

- Depending on the pest infestation, reducing populations to a manageable level varies; typically 2-5 years

- Results showed a significant impact of using the EPN multi-species approach in areas of heavy pest infestations

Rearing EPNs

Recommendations

- Rearing EPNs is a fairly simple, low-labor process

  (Testa, A. M. and **E. J. Shields.** 2017. Low Labor "in vivo" Mass Rearing Method for Entomopathogenic Nematodes. Biocontrol 106: 77-82.)

- Rearing EPNs involves careful planning; following protocols is vital to a successful rearing program

- In the rearing process it is important not to cross-contaminate EPN species throughout each stage of the rearing process. The objective is to rear a cup of pure nematodes of a given species; it is critical to apply only one species to each starter cup of wax moth larvae. If two EPN species are introduced to a single cup, one species will dominate and the production of the second species will be reduced. EPNs can later be tank mixed for a mixed application

Recommendations

- Timeline management in the EPN rearing process is essential
- Rearing EPNs should always be conducted using non-chlorinated water in all stages of rearing and application
- Temperature control is important throughout the rearing process
    - Fluctuations in temperature regimes will influence infectivity and total production of EPNs

Materials for Rearing

- 16oz starter cups (source of EPN for rearing) to supply the necessary number of infective juvenile nematodes to inoculate source cups for lab culture or field application (1-2 source cups/starter cups per species)

- Cup (16oz) of wax moth larvae;

    - Lab Culture: 2-4 cups per EPN species

    - Field Application: Number should be sufficient for the area you plan on applying nematodes (i.e. 2-4 cups of wax moth larvae per acre)

        - Minimum of 4 (16oz) cups per field/plot area

        - 2-cups per species: each 16oz cup generates 15-25 million IJs

- Fresh non-chlorinated water

Materials for Rearing

- Beakers or graduated cylinders that need to be kept separate per species
  - **Cross contamination of species can occur easily**
- Dishpan(s) capable of holding a gallon or more of liquid
- Fine mesh screen or sieve (No. 20 mesh, 841*um)*
- Coffee scoop(s) approximately 1oz. in size
- Area to store cups (16oz) of infected wax moth larvae which is dark and can be maintained at room temperature
  - *Steinernema carpocapsae* (Sc) and *Heterorhabditis bacteriophora* (Hb): 23° to 25° C (73° – 77°F)

Materials for Rearing

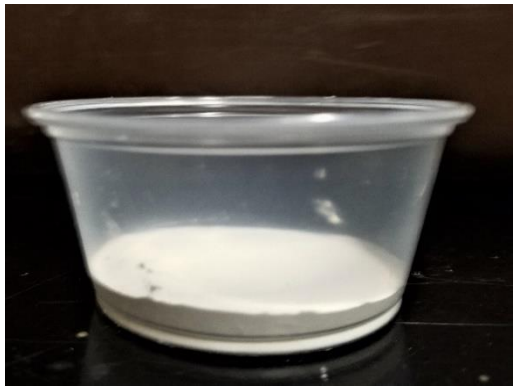
- *Steinernema feltiae* (Sf): 20° to 22° C (68° - 72° F)

  Sf is very sensitive to temperatures above 25°C, resulting in reduced reproduction and yield of IJs

- Sterilized polystyrene disposable pertri plates (100x 15mm)
- Filter papers-qualitative circles (42.5 mm)
- Plaster of-Paris pedestals
  - Pedestals are modified White traps, that are held in distilled water within petri dishes and used to collect newly emerged IJs
    - Provides an early-warning mechanism for IJ emergence
    - Collections are smaller in number which can be used to inoculate 16oz cups for laboratory rearing or starter cups
    - White traps are primarily used for laboratory rearing, however can be set-up to monitor emergence in 16oz cups designated for field release

Materials for Rearing

- White traps are made by mixing 3-parts plaster-of-Paris with 1-part water in a container to a consistency similar to a milkshake
    - Mixture should not be to watery but have the ability to be spooned out into molds
    - Plastic 4 ounce deli-cups work the best White traps should be 1/8”-3/8’ in thickness
    - “Pop” White traps out of mold >24 hours



Timing-Establishing Lab Culture

Lab Culture – If establishing a lab culture, the following timeline should be observed:

- Shields' Lab at Cornell will provide starter cups (16oz) to generate EPNs
- Order the starter cups & wax worm cups 17-21 days before the date of scheduled inoculation
- Keep wax worm cups (16oz) from bait supplier at 55-60°F (15°C) until the nematodes are applied

- Starter cups (16oz) from Shields' Lab should remain at 70° to 75°F (20° to 23°C) until nematode begin emerging from larvae cadavers, nematodes will appear as a yellow-film on edges of the cups and lid

Nematodes Emerging from 16oz Rearing Cups

Timing- Establishing Lab Culture

- Inoculate each 16oz rearing cup(s) needed per species
  - Wash each starter cup through a 20 mesh screen to generate 1 gal of water/epn solution (equals 160,000 IJ/oz., 1 oz. coffee scoop)
  - Volume of nematode solution per rearing cup ~25-30mls (30 ml = 1 oz.)
  - One 16oz starter cup will inoculate 100-128 cups for EPN release

- Add nematode solution by pouring directly onto sawdust surrounding wax worms; pouring needs to be done in a circular motion to ensure even distribution
  - Nematode solution will give a ratio of 600 IJs per waxworm in cup (cups have 250 worms)

- Label each 16oz cup lid identifying species and date of inoculation; place all inoculated cups in a semi-dark area at or slightly above room temperature (20-23°C)

Timing- Establishing Lab Culture

- Three days post inoculation, check all cups for 90-100% wax worm death
- They should look similar to these images:

Sf

Sc

- If the first attempted inoculation was not successful. You can wait for more IJs to emerge and retry inoculating new rearing cups
- After 7-days, add small amounts of water in a mist using a spray bottle, continue checking moisture in cups until yellow-film develops. Do not over moisten. Do not pour water directly into the cups

Timing – Maintenance Lab Culture

- After 4-days, select 20-30 cadavers from each rearing cup or group of cups/per EPN species that will be used for maintaining laboratory culture

  - Establish individual white traps within a single petri plate, top each pedestal with a moistened filter paper circle
  - Place ten (10) cadavers of same EPN species onto individual white traps
  - Using distilled water, fill the petri dish until trap is surrounded by water
  - Return lid to petri plate

    - ➢ Label with EPN species, date of inoculation and white trapping

- White traps are then monitored for nematode emergence

Timing - Maintenance-Lab Culture

- After 3-5 days, additional water to petri plates to prevent drying out maybe required

- Monitor petri plates for emerging nematodes
  - Visual evidence of IJs can be an indication of emergence within rearing cups
  - Collect infective juveniles by washing contents of petri plate into a container for immediate use or long-term storage

- To prepare for inoculation of a new lab culture cycle or preparing 16oz cups for rearing, take collected IJs and conduct a serial dilution
  - The minimum number of IJs for each new 16oz rearing cup is 15,000 IJs
    - Provides a ratio of 50 IJs per waxworm in cup
    - More than 15,000 IJs results in overdosing of waxworm cups which is preferable to ensure 100% infection

Timing - Observing Lab Culture in Rearing Cups

- Twelve to fourteen days post inoculation; IJs should be present on lids and sides of tubs

- IJs clusters will often be visible, similar to image on left side of the tub

- Visual confirmation indicates the tub(s) can be washed to harvest emerging IJs for new inoculations

- Nematodes (IJs) emerging from the wax worm cups should be used within 3-days; viability of IJs are at the highest-viability and decreases dramatically after 72h

Timing-Field Application

Field Application Procedures: Rearing steps for EPNs designated for field release should adhere to the steps given for lab rearing except for the following differences:

- The Shields' Lab will provide 16oz starter cups to generate EPNs upon request

- Starter cups and/or wax worm cups designated for field release on the farm need to be ordered 17-21 days prior to planned harvest and anticipated inoculation of EPNs
    - Ordering field release cups from Shields' Lab is also an option
        - An additional 7-10 days notification is required

- Wax worms cups from bait supplier need to be held at **55-60**°F (15°C) until EPNs are applied

- Starter cups (16oz) received from Shields' Lab should remain at **70-75**°F (20 -23°C) (room temperature) until evidence of emerging IJs is present

- Identify a temperature controlled area for incubation for post-inoculated field release cups

Timing-Field Application

- Rearing EPNs for field release is dependent upon species being introduced
    - Emergence using a multi-species approach needs coordination
- For field application, timing of emergence within cups after inoculation is dependent on species being utilized in application
    - 10-12d for *S. carpocapsae* ('NY01')
    - 12-14d for *S. feltiae* ('NY04')
    - 14-17d for *H. bacteriophora* ('Oswego')
- Field release cups should be ready for application based on emergence date; peak emergence of IJs will occur 14-18d post inoculation
    - It is recommended, EPNs need to be applied just prior to peak emergence for optimal establishment

Timing-Field Application

- Delaying emergence and/or viability of IJs can be accomplished by cooling the field release cups at 15°C
- Speeding up emergence by raising temperature above recommended levels is not recommended; production, viability of EPNs, and environment within cups are negatively impacted

- To avoid cross-contamination, **only** work with one EPN species at a time; complete all steps before moving to the second species

- To inoculate field release cups using starter cups from Cornell:

1) Empty contents of single species starter cup onto a 20 mesh screen; gently flush sawdust/cadaver debris using non-chlorinated water allowing IJs from debris to separate and accumulate in dishpan (generate 1 gal of water / EPN solution)

2) Remove lids from field release cups assigned to current EPN species

3) Agitate EPN solution within dishpan to prevent nematodes from settling

Timing-Field Application

4) Dip the 1oz coffee scoop into nematode solution

5) Pour contents of one scoop (apx. 1oz. – 160,000 IJs) over a single cup in a circular motion in the attempt to evenly distribute IJs, repeat process until all cups for that particular species have been inoculated. One gal of nematode solution will inoculate 100-128 cups for field release.

6) Return lids to cups, and label each cup with EPN species and date of inoculation

7) Thoroughly clean the mesh screen, dish pan(s), and coffee scoop(s) with alcohol or Clorox, rinse off with non-chlorinated water to prevent cross contamination

- Clean equipment to prevent cross contamination and repeat steps 1-7 for inoculating field release cups for the second species

Timing-Field Application

- Store all cups designated for field release in the assigned dark space
  - Do not let cups get too hot or cool (**65°F - 75°F**); death or poor infectivity will result
    - Sc & Hb: 23° to 25° C (**73° – 77°F**)
    - Sf: 20° to 22° C (**68° - 72° F**)
  - Cups will begin to smell like decay after 7-10d, **avoid** storing them in a high traffic area
  - **Do not** stack inoculated cups or store them within boxes; good air circulation is important and required

Field Application

Materials – Field Application

- **Large container** to collect nematodes from the field cups for field application (e.g., 20 gallon garbage can OR 5 gallon bucket)

- **Mesh screens (20 and 40 mesh)** to prevent wax worms and sawdust from falling into the nematode solution. Wash cans are available for use from the Shields Lab (if in NYS)

  - Aluminum or fiberglass window screen is about 20 mesh. 40 mesh screen can be approximated by doubling window screen so the opening do not match.

Materials – Field Application

- **Spray rig** – You can build your own or contact a local extension agent to inquire if one is available. If using one that has been used for any other pesticides, etc., be sure to rinse thoroughly before using for nematode application

- **Remove all screens and filters from sprayer before use**

Sprayer Requirements

Any commercial pesticide sprayer can be used to apply biocontrol nematodes with a few minor modifications:

1) All screens and filters have to be removed to allow nematode to pass through them

2) Sprayers need to be cleaned in similar manner as required when changing pesticides for applications

3) Non-chlorinated water must be used to fill the sprayer

4) Standard pressure (40-60 psi) are best for nematode survival

5) Sprayers need to apply a minimum of 50 gallons per acre (total from nozzles)

Sprayer Requirements

6) In some applications, only a portion of the nozzles are used during an application so the actual application rate of water is less

7) The best results require that a nematode-water stream is applied to the soil surface with as little as possible of the solution remaining on the plants

8) Any nozzle adjustment which sends a single stream down to the soil surface is best (i.e. Tee Jet 0015)

9) The goal is a stream of water (with nematodes) wetting the soil surface in a narrow band with separation between the bands

Ask the Shields Lab for the “Spray Guide for Application of Biocontrol Nematodes for Control of ASB” or find the link on the website

- http://www.alfalfasnoutbeetle.org/

Application Rate

- The nematode rate calculation should be conducted prior to any field application, common questions asked include:
  - How many acres will my sprayer treat at 50 gpa?
    - 300 gal sprayer/50 gpa = 6 ac)
  - What is my EPN application rate in cups/ac?
    - (4 cups/ ac)
  - How many cups need to be washed for each sprayer fill?
    - 6 ac x 4 cups/ac = 24 cups need to be washed per sprayer fill

Application Protocols

- Bio-control nematodes or EPNs are applied to the soil surface and require some shading from sunlight because they are UV sensitive until they enter the soil

- Applications should be made late in the day (after 6pm), after dark or during cloudy and/or rainy days to minimize nematode death from intense UV sunlight. Avoid applying them to hot soil surfaces

- Bio-control nematodes must be removed from the sawdust/wood chips found within the wax worm cups

- Attach modified trash can lids with mesh screen and flashing to 20-gallon trash can (coarse and fine mesh lids are included in set)

Application Protocols

- Dump contents of multiple 16oz wax worm cups onto the coarse wire screen (20 mesh) and wash nematodes through into can with a large volume of non-chlorinated water. Maximum number of cups/washing = 16

  - 20 mesh window screen also works
  - If applying multiple EPN species, number of 16oz cups washed must be equally distributed within wash can (e.g. 8 Sc & 8 Sf)

- Volume of EPNs collected inside can must minimally equal 1gal per 16oz cups washed
  - Washing 16 cups should generate 16-20 gallons of EPN solution
  - Fill the garbage can with additional water to near capacity (20gal) once desired volume has been reached

Application Protocols

- The collected nematode solution needs to be poured through a second filter screen (fine = 40 mesh) to remove finer debris which will clog nozzles

- After the second screening, the solution containing nematodes is ready to be dumped/pumped into the spray tank for application

- Fill the rest of the spray tank with non-chlorinated water prior to each application run

Soil Bioassays for EPN Presence

Background or Purpose

Thirty to 60 days after application:

- Soil cores can be taken and tested to determine whether the nematodes have established
- Because **each** nematode species occupy different depths in the root zone, each soil core will be divided
- Soil from the upper two inches will be placed in a small cup, and soil from 2-6 inches will be placed in a larger cup
- Soil will be exposed with healthy wax moth larvae
    - Active nematodes within soil sample will kill healthy wax moth larvae providing evidence of presence

Materials - Field Bioassay

- **Soil Probes** to collect soil samples from field sites to determine presence or lack thereof of EPNs in the soil
  - Oakfield 36” Tube Sampler Soil Probe Model L
  - AMS 40” One-piece Step Probe
- **Plastic Soufflé Cups** to place soil collected from field
  - Fabri-Kal 4oz Polystyrene Cups (PC400)
  - Newspring 8oz translucent round deli container cups (YL2508)
- **Plastic Cup** lids to seal soil collected from field
  - Fabri-Kal Clear Pet Plastic Cup Lids (XL345PC)
  - Newspring 8oz translucent round deli container lids (YL2508)

Materials - Field Bioassay

- **Garmin GPS** handheld navigator used to mark and record soil sampling location for each sample point
    - Garmin™ GPSMAP 64st or equivalent to download the GPS data

- **PVC Table** or similar set-up to allow mobility throughout sampling area with sample materials and collected samples

- **Garbage bags** or boxes to store and transfer the collected soil samples back to laboratory
    - In warmer conditions, ice chests may be necessary
        - ➢ Higher temperatures will kill the nematodes in the soil sample containers

Field Bioassay Preparation

- In preparation for collecting soil samples from the field, creating sets of collection bags proves to be the most efficient way to go
    - Pre-label the 4oz and 8oz lids that will be used in the field assay
        - Label using a letter identifier followed with a numbering sequence 1-100 (e.g. A1-10, A11-20, or 1A1-10, 1B11-20)
        - Rubber band matching 4oz and 8oz lids together for each set
        - To avoid confusion different lettered sets should be created
- In a large garbage bag or box, place lids from one set (1-100) of lids along with 4oz and 8oz deli cups (1-100 for each size), also include two empty garbage bags that will be used to collect and transfer samples
- Print off field maps identifying the area in which the assay will occur
    - Include GPS points, highlighted landmarks, or label for ease of entry

Field Bioassay Protocols

- The number of samples taken in each sampled field/plot is proportioned to the acreage of the field/plot
    - EX: (in research mode)in each 0.25 acre plot typically 25-50 samples are collected
    - To provide a representative picture of the EPN population, sampling locations are distributed evenly throughout each field/plot
        - Large field applications only require a lower number of random samples
- Record location of sample using GPS™ handheld
    - Downloading of marked locations allows the ability to map and observe areas of nematode activity

Field Bioassay Protocols

- Using soil probe, remove a 1” diameter x 6” or greater long soil core from ground

Field Bioassay Protocols

- Separate the top 2” of the soil core from the lower 4”+ of the soil core
  - Place each portion in the appropriate cup
  - 0-2” in 4oz cups
  - 2-6” in 8oz cups

- Soil cores are divided in this manner to isolate EPN species during the field assay
- Once row or set of cups are filled, seal cups with pre-labeled lids securely for transfer from field to laboratory for assessment

Materials - Laboratory Bioassay

- **Kitchen forks** that will be used to crumble/breakup soil samples inside containers; number of forks should be 3-6 dozen
    - More efficient to have large number of forks available to speed up processing of soil containers
- **Hot plate** to heat water inside 1000ml beaker for sterilizing of forks used to break-up individual soil containers
    - Do not boil water – should be able to grab forks out of beaker
- **1000 ml beaker** filled to 500 ml with alcohol for an additional sterilization of forks
- **Galleria** (waxworm) larvae
    - Number of larvae required depends on number of samples to process
        - One 16oz container of waxworms holds 250 worms, enough to evaluate 16 samples; 6 wax worm cups for every 100 samples

Materials - Laboratory Bioassay

- **Galleria** (waxworm) larvae continued:
  - 5 galleria larvae for top 0-2” sample
  - 10 galleria larvae for bottom 3-7” sample
- **Number 4 or 5 sieve** to separate waxworms from sawdust within cups
- **Container** or empty cups to hold separated worms for dispersal
- **Spray bottle** filled with non-chlorinated water
- **Incubation** of cups should be conducted at room temperature (70°-75°F), dark area preferred but not necessary
- **Labeling tape** for storage to identify sample site and assay set-up date

Sample Storage - Laboratory Bioassay

- Soil cores are returned to the laboratory inside deli-containers and bio-assayed using a standard technique with wax moth larvae as an indicator for the presence of biocontrol nematodes in the sample
    - Soil that was dry when sampled should be evaluated within 3-5 days
        - Prolonged delay for evaluation may result in soil drying out and any present IJs dying
        - Misting of each soil sample and reclosing can extend the life of the sample in storage for an additional 1-2 weeks
    - Soil that was damp or wet when sampled can be stored at 10°C (50°F) for 2-3 weeks

Sample Storage - Laboratory Bioassay

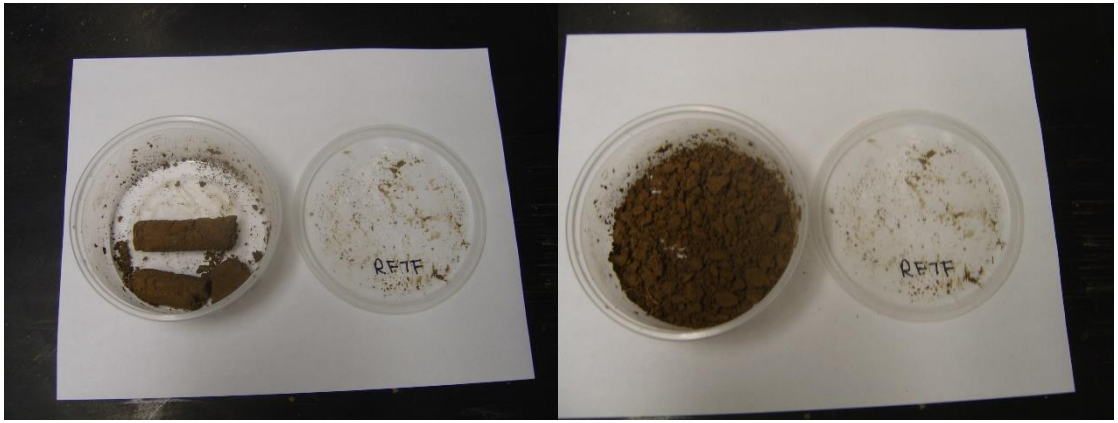
- Considering prior statements, samples from a field site or rep should be set up within a couple days when possible
  - If processing of cups cannot be conducted within 10 days, samples should be stored at 10°C (**50°F**)
  - Once cups are processed and larvae added; evaluation for presence of IJs needs to be conducted between 6-8 days after host exposure

Preparation - Laboratory Bioassay

- Sterilization of materials used for assay
    - Turn on heat source and place beaker with approximately 400mls of water to heat being careful not to boil; able to retrieve forks to the touch
    - Place beaker with alcohol near heat plate for additional sterilizing
- Prior to beginning a bioassay, deli-containers need to be separated by field identification – top and bottom soil samples (split sample sections)
- Take samples from one site and begin to remove lids
    - Once lids removed, gently break all clumps of soil up using a single sterilized fork per sample cup; do not smash fork as it may kill any nematodes within the sample

Preparation - Laboratory Bioassay

- Soil within cups does not need to be fine; can have small clumps of soil, remove any stones or debris (e.g. roots)



Before Preparation After Preparation

- Mist soil cups with a spray bottle two-three times based on the moisture level within the sample cup

- Add wax moth larvae to cups and then close lids
  - 5 wax moth larvae to each 4 ounce deli-container
  - 10 wax moth larvae to each 8 ounce deli-container

Preparation - Laboratory Bioassay

- Close lids and place cups inverted on a bench or shelf for a minimum of 7 days; room temperature and darkness for at least 14h per day

Evaluating Cups - Laboratory Bioassay

- A minimum of 7d after exposure, remove cups from storage and check each container (4 & 8 ounce) for death caused by nematodes

  - Galleria will turn tan when killed by Sc ('NY001')

  - Galleria will turn dark brown when killed by Sf ('NY04')

Evaluating Cups - Laboratory Bioassay

- Galleria will turn brick red when killed by Hb

- Record results for each container, including:
  - Number alive and number dead,
  - Death by nematode species and/or by other cause

Evaluating Cups - Laboratory Bioassay

- If you are unsure the wax moth larvae is dead as a result of an EPN, white trap the cadaver and check for emerging juveniles 7 to 10 days later
    - Additionally verification of EPN species can be conducted by dissecting out the adult males and identifying EPN based on the male spicule head or DNA analysis
- Dispose soil and cadavers by dumping contents of each container into a bag or tub; freeze or autoclave before throwing into trash
    - 8 ounce containers can be washed and reused
    - 4 ounce containers can be tossed out

EPN Persistence Preservation Protocols

Preserving Persistence Genetics

EPNs reared continuously in the laboratory adapt to those laboratory conditions and lose traits which help them survive under field conditions. The Shields' Lab adheres to the inoculative approach requiring EPNs to have the ability to retain their adaptation for field survival. Several approaches are recommended that can be utilized to help retain genes within the laboratory culture environment

- Continuous culture of EPNs in the laboratory or commercial production facility utilizes only the immediately infective portion of the population (less than 40%)

Preserving Persistence Genetics

- Continuous culture utilizing only these immediately infective IJs
  - Narrows the gene pool to those individuals who do not carry the coding for delayed infectivity
  - Reduces the associated benefit of the ability to persist across unfavorable conditions
- Loss of field persistence for native populations of Sc, Sf, and Hb occurs in less than 15 generations in the laboratory

Field Re-isolation

- Re-isolation of native and/or introduced populations from areas previously inoculated with EPNs
    - Isolations are used to completely restart laboratory cultures
    - Protocols require isolation of cultures every 2-years
- Initially the new culture cycles will produce reduced yields of IJs until adaption to laboratory conditions and rearing host are made
- Mixing of new genetic material from re-isolated individuals with continuing laboratory cultures is not recommended and does not successfully retain the persistence genes/field adaptation
    - Results have shown this practice only delays the loss of persistence

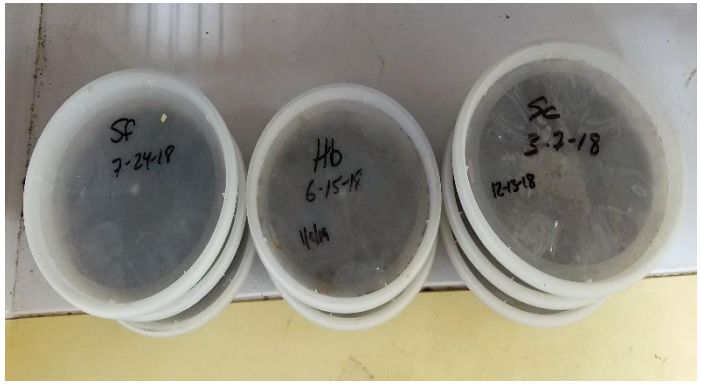
Wild Populations

- A second strategy utilized by the Shields' Lab is creating a "backup plan" to maintain EPN populations adapted to NYS conditions
    - Referred to as natural storage areas
- The Shields' Lab utilizes surrounding ornamental plants and grassy areas, on Cornell campus which are invaded by insect hosts
- Selected individual areas are isolated and have been inoculated with individual populations of NY native species of EPNs
- Subsequent sampling is required to document persistence of the inoculated populations/species in these "wild" areas

Laboratory Culturing

Laboratory culturing methodology to help preserve the persistence genetics has proven to be an effective strategy

- Fill multiple 16 oz. deli-cups with 200g of moistened autoclaved loam soil
    - Cups will require fitting lids to retain moisture for long periods
- For each species in EPN culture
    - Inoculate 3-6, 16 oz. deli-cups with 1,000 IJs per cup
    - Secure lid and label with date (e.g. MM/DD/YYYY)



Laboratory Culturing

- Store cups at room temperature (20-22°C) on a dark shelf in a cabinet

- Multiple containers are established monthly per species – 12-24 month period
- To maintain the laboratory culture, every 3mo, remove containers from storage for each EPN species
    - Cups removed should represent a time period of 6-18 month or longer
    - Within each container, place 5 wax moth larvae
    - Return cups with wax moth larvae to separate storage area for 5-7d

Laboratory Culturing

- After 5-7d, remove cadavers infected from containers
  - Avoid cross contamination by removing one EPN species at a time
  - Keep cadavers separated by inoculation date on lid surface

- White trap cadavers by species by selecting 1-2 cadavers from each date and grouping together for IJ emergence; creates a mixture of IJs from across the stored soil time period
  - Establish individual white traps within a single petri plate, top each pedestal with a moistened filter paper circle
  - Place ten (10) cadavers of same species onto individual white traps
  - Using distilled water, fill the petri dish until trap is surrounded by water

Laboratory Culturing

- Return lid to petri plate
  - Label with species, date of cadaver retrieval and white trapping

- White traps are then monitored for nematode emergence

- Mark lids of used storage containers with retrieval date(s) and return to storage; once there are no more deaths from EPNs, discard containers
  - Typically 12-18mo for Sf and HB, 3-6mo for SC

- By adding new soil containers each month, you are creating a continuous array of “aging” soil samples with IJs becoming infective at any point in time
- Technique has been successful in retaining the phased infectivity trait, but does little to prevent adaptation to rearing under moderate temperatures

Contact Information

Bait Suppliers	Cornell Cooperative Extension Agents	Cornell Shields Lab
Knutson's **tom@knutsondecoys.com** **1-517-592-2786**	Jefferson/Lewis Counties Michael Hunter Meh27@cornell.edu 1-315-778-8602	Tony Testa Cornell Entomology Department At28@cornell.edu 1-607-591-1493
Speedy Worm **http://speedyworm.com** **sales@speedyworm.com** **1-320-762-8247**	St. Lawrence/Franklin/ Clinton/Essex Counties Kitty O'Neil Kao32@cornell.edu 1-315-854-1218	Elson Shields Cornell Entomology Department Es28@cornell.edu 1-607-279-1849
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