

*Proceedings of*  
**BENEFICIAL NEMATODE**  
**WORKSHOP**

*Application in Greenhouse, Nursery, and Small-Fruit  
Operations*

Edited by P. W. Gothro



INTEGRATED PLANT PROTECTION CENTER

IPPC Publication 900, September 7, 2000

*North Willamette Research and Extension Center*

*Aurora, OR*



**OREGON STATE  
UNIVERSITY**



# Oregon State University

*Proceedings of*

## **BENEFICIAL NEMATODE WORKSHOP**

Application in Greenhouse, Nursery, and Small-Fruit  
Operations

Edited by  
Peter Gothro  
Department of Entomology  
Oregon State University  
Corvallis, OR 97331-2907

September 7<sup>th</sup>, 2000  
North Willamette Research and Extension Center  
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## **CONTRIBUTORS**

R. E. Berry

Department of Entomology, Oregon State University, Corvallis, OR 97331-2907

S. R. Booth

Washington State University Research Unit, Long Beach, WA 98631

P. W. Gothro

Department of Entomology, Oregon State University, Corvallis, OR 97331-2907

E. E. Lewis

Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0319

R. Rosetta

North Willamette Research and Extension Center, Oregon State University, Aurora, OR 97002-9543

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# AGENDA

- 8:00 – 8:30     **Registration**
- 8:30 – 9:30     **Biology, Selection, Handling and Application of Entomopathogenic Nematodes**  
E. E. Lewis
- 9:30 – 10:15   **Application of Beneficial Nematodes for Control of Fungus Gnats**  
P. W. Gothro
- 10:15 – 10:30   **BREAK**
- 10:30 – 11:15   **Use of Entomopathogenic Nematodes to Manage Insect Pests in Small Fruit Crops**  
S. R. Booth
- 11:15 – 12:00   **Control of Root Weevils in Nursery Crops with Entomopathogenic Nematodes**  
R. E. Berry
- 12:00 – 1:00    **LUNCH**
- 1:00 – 3:30     **Demonstrations – Hands On**  
Calculating Application Rate  
- Checking Viability  
- Calculating Quantity of Nematodes Bought  
- Calculating Quantity of Nematodes Needed
- Application of Nematodes with Different equipment: spray tanks, irrigation/fertilizing equipment, etc.
- 3:30             **End**

## PREFACE

Entomopathogenic nematodes are currently being used to a limited extent in greenhouse, nursery, and small fruit operations to better manage insect pests. Key to their wider use will be a better understanding of nematode biology, how they should be handled, how they may be applied, and where they may be obtained. The object of this workshop and of this book are to provide the potential user of entomopathogenic nematodes a place to find answers to questions that may arise when working with these organisms.

The use of pesticides in agriculture is receiving much scrutiny these days, from regulatory agencies and the general public. More and more, concerns of worker safety, water quality, spray drift, and effects on non-target organisms are subjects that must be addressed by farm managers and pest control specialists when dealing with pest problems and the application of materials to manage these problems. In conjunction with the advent of more selective pesticides, entomopathogenic nematodes give agriculturalists another tool with which they may better manage pest problems. As with any tool though, the importance is knowing when and how to use it in order to achieve the desired result. In the way a woodworker knows when to use a spokeshave versus a drawknife, the successful use of entomopathogenic nematodes are no different, as the user must know what species of nematode to use for a given pest.

Nematodes are, by no means, a be all – end all for pest problems. There is no substitute for scouting to determine if there is a pest, and if so, if the pest would be susceptible to nematode ‘attack.’ Knowing about the target pest and its biology, as well as the range of entomopathogenic nematodes available for use against the pest are also important. Knowing what compounds are compatible for use with nematodes or how long to wait between application of incompatible chemicals and a nematode application can mean the difference between success of a treatment or a loss of time, energy, and other resources.

This workshop was made possible through a grant from the Integrated Plant Protection Center (IPPC) at Oregon State University, for which I am grateful. Furthermore, I would like to thank the workshop speakers, Drs. Ralph Berry, Steve Booth, and Ed Lewis, for their time and energy to help make this workshop ‘work.’ Without the helpful guidance, suggestions, and extensive rolodex of Robin Rosetta, the different components would not have come together so smoothly. Last, though definitely not least, the skills of Heidi, Paula, Geoff, and Gideon of NWREC were certainly key to this workshop coming together.

# **Biology, Selection, Handling and Application of Entomopathogenic Nematodes**

E. E. Lewis

Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0319

Beneficial insect-parasitic (entomopathogenic) nematodes have been available as a microbial insecticide for nearly 20 years. Many nursery and landscape professionals have used entomopathogenic nematodes to manage insect pests with varying degrees of success. One success story has been the control of the grub stage of the black vine weevil, which infests nursery crops. In fact, entomopathogenic nematodes are one of very few effective treatments for the soil-inhabiting grub stage of this pest. However, entomopathogenic nematodes have failed to control insect pests on some occasions. Why is the level of control provided by these nematodes unpredictable? My goal is to address key issues that influence success when these nematodes are used as biological control agents.

Most of the time, dissatisfaction with entomopathogenic nematode treatments result from a lack of knowledge about their biology, ecology, storage requirements, and proper application conditions. When entomopathogenic nematodes are formulated, packaged and sold as microbial insecticides, it is often forgotten that they are actually living animals with strict requirements to stay alive and remain able to infect insects.

Professionals who attend trade shows and grower meetings where I speak benefit most often from learning basic principles of entomopathogenic nematode biology and ecology. In a short article or talk, it is impossible to address all of the situations a landscape or nursery professional would potentially encounter in a season. However, armed with accurate information about nematode biology, decisions about when and where to use entomopathogenic nematodes can be made by users on a case-by-case basis. At the very least, an understanding of entomopathogenic nematode biology will enable you to ask pertinent questions when you call the nematode supplier or extension personnel for assistance. The best approach, as we have in this workshop, is a combination of basic information and specific case histories.

Currently, there are two genera of entomopathogenic nematodes; *Steinernema* and *Heterorhabditis*. The nematodes in these genera have similar life histories, but are not closely related. *Steinernema* and *Heterorhabditis* nematodes enter the hosts as infective juveniles. Each infective juvenile carries bacteria in the gut, and releases the bacteria into the body cavity of the insect after penetration. The bacteria kill the host in about 48 hours.

*Heterorhabditis* nematodes carry bacteria in the genus *Photorhabdus* and *Steinernema* nematodes carry *Xenorhabdus* bacteria.

Entomopathogenic nematodes are sold when they are in the infective juvenile stage. This is the third of six life stages (all nematodes have an egg, four juvenile stages and the adult stage), and is the only stage that survives outside the insect host. The infective juvenile nematode does not feed, mate, or develop outside an insect host. When they are applied to control insect pests, they are unable to supplement their energy reserves, so they must find and infect a host before this energy is used up.

When we apply entomopathogenic nematodes, we release the infective juvenile into the area we want to protect. The nematodes search out an insect host and enter it through the mouth, anus, spiracles, or sometimes by making a hole in the cuticle. Inside the insect, the nematodes release their bacteria. The bacteria develop by degrading the insect tissue, and kill the insect within two days. The nematodes develop by feeding on the bacteria, reproduce for one to three generations within a host, and about 10 days later, up to 500,000 infective juveniles emerge from the insect host ready to infect a new host.

**“Rules of thumb” when considering entomopathogenic nematodes for insect pest management.**

**Entomopathogenic nematodes are soil organisms.** This means that they are most effective when used against insects in protected habitats. Soil insects (black vine weevil grubs, white grubs, etc.) and boring insects (dogwood borer, iris borer, etc.) have been controlled successfully by entomopathogenic nematodes. Attempts to use them against foliar insects have seen very limited success.

Entomopathogenic nematodes do not tolerate either dry conditions or direct sunlight. When treating soil in potted plants, it is essential to water before applying nematodes. Watering after application can help, but be careful not to wash the nematodes out of the pot. To avoid problems with UV light, apply nematodes to outdoor areas at the end of the day to give the nematodes time to move into the soil during low light levels. If nematodes are applied to landscapes, irrigate immediately after application.

**All nematodes are not the same.** Some species of entomopathogenic nematodes are effective against insects that live close to the soil surface, and others are equipped to infect insects deep in the soil. *Steinernema carpocapsae*, the most widely-available entomopathogenic nematode, is effective when used against surface-dwelling insects, but is ineffective against insects deep in the soil, like white grubs. For insects deep in the soil *Heterorhabditis bacteriophora* or *Steinernema riobrave* are most effective. The reason behind this differential efficacy is related to the way the nematodes search for hosts. *Steinernema carpocapsae* infective juveniles search for hosts by standing on their tails and elevating the front 95% of their

body from the substrate. They stand perfectly straight and still for hours at a time, waiting for a host insect to walk over them. When the host comes into contact with the nematode, the nematode sticks to the host and then enters the body cavity, as described above. Obviously, if the nematode is standing on its tail on the soil surface, the odds of it finding an underground insect host are slim. Most other nematode species move through the soil in search of insect hosts, and are not associated with the soil surface. These species will encounter underground insects and infect them.

Other differences among entomopathogenic nematode species include host specificity, activity levels in cool temperatures, and tolerance to warm temperatures. This information, along with the entomopathogenic nematode species in a product should be listed on the label. If this information is not on the label, contact the manufacturer and ask. The wrong choice of nematode species will often lead to poor results.

**Product shelf life is limited.** The first and most important thing to remember about transporting, handling and storing entomopathogenic nematode products is that the nematodes are living organisms and need to be treated as such. The infective juvenile stage is not considered a “resting stage”. Unlike other microbial insecticides (e.g., Bt products, viruses or fungi), entomopathogenic nematodes use up their limited energy reserves even when they are formulated. A new breakthrough in entomopathogenic nematode formulations, a dispersible granule, has extended the shelf life of entomopathogenic nematode products to up to six months. Most formulations for entomopathogenic nematode products, including the dispersible granule, slow metabolism but they do not stop it entirely. Therefore, products have limited shelf lives compared with chemical insecticides and many other microbial insecticides. Granular formulations may appear very similar to chemical pesticides and it is tempting to treat them as chemicals. However, chemical insecticides are not affected by many environmental conditions that are lethal to entomopathogenic nematodes. In short, entomopathogenic nematode products require specific conditions during transport and storage to remain viable. Check label recommendations and do not use product that is too old. You may be applying dead nematodes.

Assessment of a product’s viability after purchase is not difficult, has minimal equipment requirements and can protect the end user from applying dead entomopathogenic nematodes.

Equipment needed:

1. An inexpensive dissecting microscope or hand lens with at least 15X power
2. A good light source
3. A black or other dark colored surface
4. A clear, shallow dish for the examination (a Petri dish is ideal)
5. Water

To check for nematode viability, nematodes must be first released from their formulation. The methods for this are variable, depending upon what kind of formulation is purchased. Remember that the nematodes are, for the most part, about 0.5 mm long, so you need only check a very small amount of the purchased material. For example, a single granule will contain hundreds of individual nematodes, so a few granules are certainly enough to get a rough idea of viability.

### **General Methods**

1. Take a small amount of formulated product.
2. Prepare the sample for application following the instructions on the label and put the nematodes and water in the examination dish.
3. Wait for the nematodes to revive from formulation. This duration will be written on the product label as the time to wait between preparing the product and application.
4. Put the examination dish on the dark-colored surface and shine the light from the side, so you see white nematodes with a dark background. If the nematodes are difficult to see due to the formulation material making the water cloudy, dilute the sample.

Some formulations may require the addition of an “activator” to release nematodes from a gelatinous matrix. In this case, it may be necessary to check for viability after putting together the tank mix. After the allotted time for activation has passed, all nematode species, except for *Steinernema carpocapsae*, should be moving in a sinusoidal (“S”-shaped) manner. *S. carpocapsae* infective juveniles will move when prodded using a pin or sewing needle. At rest, they assume a typical J-shape. Dead nematodes appear to be straight, do not move and are often clear. Another indicator of nematode viability is the density of lipid (fat) throughout the body. The nematodes should look “solid white” and not have the appearance of having bubbles within the body. These “bubbles” are actually water droplets that have replaced used-up energy reserves.

**Preventative treatments with nematodes do not work.** When using entomopathogenic nematodes, monitoring pest populations is key to success. The life expectancy of entomopathogenic nematodes in the field after application is not well-known, so apply them when hosts (the target pests) are present.

**The standard field application rate for entomopathogenic nematodes is 1 billion nematodes per acre.** When scaled to a per-pot basis, this works out to 20,000 nematodes for a 12 inch pot. Manufacturer recommendations may differ from this figure. Always follow the manufacturer's recommendations.

**Most entomopathogenic nematodes available for sale will not be effective at soil temperatures cooler than 60° Fahrenheit.** Before applying, the soil should be warm enough for the nematodes to be effective.

Finally, as with all IPM programs, record product performance and customer satisfaction. Keep track of application techniques that work and don't work, and your effective use of entomopathogenic nematodes will improve from year to year.

# Application of Beneficial Nematodes for Control of Fungus Gnats

P. W. Gothro

Dept. of Entomology, Oregon State University, Corvallis, OR 97331-2907

## OVERVIEW

Fungus gnats and shore flies, while not very big, may have a large effect on the success of a greenhouse/nursery operation. This is because they may: 1) feed on the tender roots/root hairs of seedlings (fungus gnat larvae); 2) transmit (vector) plant pathogens like *Verticillium*, *Pythium*, *Cylindrocladium*, *Sclerotinia*, and *Theilaviopsis* (adult fungus gnats and shore flies); or 3) indirectly injure ornamentals due to fecal spotting (shore fly adults). Developing a management program for these pests in the past often relied heavily on chemical compounds, but in the last several years, growers have been turning to entomopathogenic nematodes as a management tool. Success of these programs involves the correct selection of beneficial nematode, proper use of the nematode, and adequate monitoring.

## Major Components

As with any pathogen/disease, there are three key components/conditions to the system it operates in:

1. Susceptible host (target pest).
2. Competent pathogen (healthy, correct 'stage').
3. Conducive environment (for host and pathogen).

These three components are referred to as the "Disease Triangle", and if any of these conditions are not met, there is no disease (or infection/control/success).

Regarding fungus gnats, the most susceptible stages are the immature forms (larvae and pupae), primarily because they are 'soft-bodied', and are the stages present in the soil/potting mix. It has been reported that adults are able to be infected as well, though we would expect at a much lower rate than the larval and pupal forms. Shore flies, on the other hand, because they feed primarily on algae, are usually not present in the potting mix. It is important to understand the biology of fungus gnats, namely that the immature forms are below the surface of the soil feeding on roots, though they may be between the roots and plug in liners.

The selection of a competent pathogen, or nematode in this discussion, is a factor that may be overlooked at times. While there are definitely many nematodes that will infect and kill fungus gnats, some types of nematodes are more appropriate for use than others. The most widely produced nematode,

*Steinernema carpocapsae*, will kill sciarids, but the nematode *S. feltiae* has been found to do the job better. This may be in part due to the fact that *S. carpocapsae* was originally isolated from a lepidopteran (moth) while *S. feltiae* was isolated from a dipteran (fly). Another factor to consider when selecting a nematode is the genus, *Heterorhabditis* or *Steinernema*, and possibly the particular strain. It is not very likely that most nematode producers will have different strains available any time in the near future.

A conducive environment for the system is often factor that is easy to overlook. We might find ourselves identifying the target, the fungus gnat, selecting the correct nematode, *S. feltiae*, and then applying the nematodes as we might a conventional pesticide. We need to take into account the abiotic, or physical, factors of the environment. Research has shown that nematodes are inactivated by sunlight/uv (it dries them out, etc.), and many nematodes are ineffective above 25-26° C (77-79° F). Equally important is ensuring that there is adequate moisture before, during, and after nematode application to maximize the chance of successful infection.

## **ADDITIONAL CONSIDERATIONS**

While keeping in mind the three components of the disease triangle, there are other factors to keep in mind as well. One of the most important other factors are what other pest management tools are being used in the area where nematodes are to be applied? Was the area treated with a nematicide, or will it? If so, what is the time interval between the applications? What other compounds have been, or will be applied to the area nematodes are to be used? While some compounds are very toxic to nematodes, they may be safely used in the same area if there is separation over time.

The condition of the application equipment is also a concern, though good spray etiquette should ensure there are no harmful residues left in the system. Often overlooked are the filters before the spray nozzle. If used, they should be 50 mesh or larger. On the other hand, if a 'wand' is used to drench plants, make sure the line is 'charged' with nematodes before starting application. It is also important to keep the nematode solution agitated to prevent settling and ensure even distribution. When mixing the nematodes for application, it is important to use cool water as water temperatures as low as 80° F could inactivate or kill the nematodes, whereas cooler temperatures will only slow the nematodes down and not harm them.

Monitoring is an essential component of any pest management program, and if implemented properly, it should pay for itself in a short time. With fungus gnats, yellow sticky cards are often used to monitor (and to limited extent, control) adults. By inspecting these as part of an IPM program, it may be possible to stop a problem before it starts, thereby saving money.

## **CONCLUSIONS**

As with any pest management program, keep records of what tools (nematodes) were used and how they were implemented to help develop a system that will work for your operation. Ask suppliers/producers about their products and how they may change over time: Are other strains, isolates, or species available more suited to your operation? Does a producer offer 'custom strain' production? What about cost?

Always keep in mind the three parts of the disease triangle when using nematodes. Also bear in mind that nematodes are not a be-all end-all for fungus gnat problems, but rather another tool in the toolbox a pest manager has to use against this and other pests.

# Use of Entomopathogenic Nematodes to Manage Insect Pests in Small Fruit Crops

S. R. Booth

Washington State University Research Unit, Long Beach, WA 98631

## I. INTRODUCTION

Small fruit crops in general possess some traits that may enhance the potential of entomopathogenic nematodes against subterranean pests, so this section presents some general considerations and methods for the application of entomopathogenic nematodes against subterranean insect pests in small fruit crops. Nevertheless, small fruit crops are diverse in terms of cropping type, growth requirements, and management tactics, so some specific protocols are noteworthy. These considerations and tactics are presented for cranberry, blueberry, caneberry (red raspberry, blackberry, boysenberry, loganberry, marionberry, etc.), grape, and strawberry.

## II. KEY INSECT PESTS OF SMALL FRUIT

- A. Numerous, diverse, and distributed among all common terrestrial taxonomic orders.
- B. Key pests are often the same despite the crop.
- C. Can be categorized according to functional group or feeding guild. As in other crops, soil-dwelling root-feeding pests of small fruits have greatest potential to be controlled by entomopathogenic nematodes.
  1. Black vine weevil (*Otiorynchus sulcatus*), strawberry weevil (*O. ovatus*), obscure root weevil (*Sciopithes obscurus*), and wood's weevil (*Nemocestes incomptus*) -- common in many small fruit crops in most northern and some southern temperate growing regions.
  2. Scarab grubs—fairly ubiquitous, but less common in the PNW.
  3. Cranberry girdler (*Chrysoteuchia topiaria*), (e.g., sod webworm) -- severe pest of cranberry in most Northwest growing regions.
  4. Grape root borer (*Vitacea polistiformis*) (Sesiidae) -- common in American Midwest and southeast.

## III. THE POTENTIAL OF ENTOMOPATHOGENIC NEMATODES IN SMALL FRUIT CROPS

- A. Potential could be large—small fruit crops are often perennial and grow in relatively undisturbed soils
- B. Although most field studies have demonstrated good efficacy, entomopathogenic nematodes have not been well implemented in most pest management programs in small fruits.

- C. Many field studies have been limited to smaller plots, featured non-traditional application techniques, and were conducted by a small cadre of forward-thinking researchers.
- D. In Pacific Northwest small fruit crops, the success of the most commonly commercialized nematode, *Steinernema carpocapsae*, has been modest because soil temperatures are often below its threshold for activity (15° C) during optimum application times.
- E. Difficulties in formulation and marketing have slowed implementation. Smaller scale and less expensive production techniques, and the research and development of effective as well as producible strains may be more encouraging.

#### IV. MONITORING FOR PESTS

- A. Subterranean pests in dense soils can be sampled using a large diameter (10 cm) soil corer or golf cup cutter.
- B. Sandy soils can be sampled with shovels and sieved through screens to detect large larvae or cadavers. Caneberry plantings are less dense than matted crops and peripheral roots and nonfruit-bearing primocanes can be sampled without damaging the main crown or floricanes. Each side of a trellised row receives sunlight at different times of the day, so soil should be sampled from both sides or consistently from the same side.
- C. Root weevil larvae do not float in water and some soils contain a lot of debris, so they must be sorted by hand. In strawberry, the entire plant and the surrounding soil should be carefully examined, as larvae are often inside the crown.
- D. Adult root weevils are nocturnal but usually fully active within an hour after sunset. In cranberry, weevils can be sampled with a sweep net. In caneberry, they can be dislodged onto a tarp by vigorously shaking the trellis 3 times and then counted using a flashlight. In grape, Cone et al. (1990) suggested sampling weevils in traps hung on the trellis wires. In strawberry, weevils can be directly observed on the foliage with the aid of a flashlight.

#### V. FACTORS DETERMINING APPLICATION TECHNIQUES

- A. Characteristics of the pathogen
  - 1. predatory behavior
  - 2. activity temperature threshold
- B. Characteristics of the pest
  - 1. seasonality / lifestage susceptibility
  - 2. distribution (uniform vs patchy)
  - 3. rate of increase (ability to disperse as well as reproduce)
- C. Characteristics of the crop
  - 1. value
  - 2. age (annual vs bi- or tri-annual)

3. soil type and structure
  4. crop structure (plant height, % soil cover, trellised)
  5. soil moisture and irrigation method
- D. Specific Factors by Crop
1. Cranberry
    - a. Two distinctive crop characteristics:
      - (1) Pesticides are commonly applied through the set sprinkler system
        - (a) untreated areas can be covered with tarpaulins, but they must be removed immediately after treatment.
        - (b) Alternatively, plug or turn off nozzles of conflicting sprinklers provided system water pressure and the delivery at other sprinklers are not affected.
      - (2) Crop's extreme sensitivity discourages destructive sampling, making it difficult to assess nematode efficacy or persistence.
    - b. Characteristics of pest and pathogen
      - (1) Due to dense structure of both soil and crop, entomopathogenic nematodes may not move as much in cranberry as in other crops. Both *S. carpocapsae* and *Heterorhabditis bacteriophora* moved only 4 cm in 14 days in a cranberry bog on the east coast, whereas a small percentage of the latter species (HP88) moved 30 cm in 60 days in a west coast cranberry bog. A similarly low percentage of inoculated *H. bacteriophora* moved more than a meter during a 10 month period in a very sandy bog.
  2. Caneberry and grape
    - a. Crop characteristics:
      - (1) Farms and vineyards are often large and crop rows (alleys) are separated by trellis and wires, so pest infestations may be more patchy and difficult to locate than in other crops.
      - (2) Conventional application is usually by air blast sprayers that may be harmful to entomopathogenic nematodes. (Studies in apples have demonstrated that nematodes can survive air blast application).
      - (3) Chemigation practices using above ground and below ground trickle and drip systems are becoming more common.
    - b. Pest characteristics: Perhaps more than in other crops, root weevil infestations in caneberry are located at field edges and usually extend along trellised rows rather than across them.
  3. Strawberry
    - a. Crop characteristics:
      - (1) Perennial, often cultured as an annual crop in warm locales, and bi- or tri-annuals in cooler regions. A thick understory of foliage and duff develops by late season and overwintering plants are often protected by mulching hills with straw or black plastic. These ground layer habitats promote microbial organisms, but fumigation between plantings suppresses them.

- (2) Size and location of most infestations are easily discerned in short, fast growing strawberry plants. Strawberries grow in a variety of soil types.
- b. Pest Characteristics:
  - (1) In the Pacific Northwest, the root weevil complex in strawberry typically consists of 3 -- 4 spp which are very difficult to distinguish as larvae, aside from size. Most species also have overlapping lifestages, further confounding identification.

## VI. APPLICATION TIMINGS

### A. Pest Phenology:

#### 1. Black vine weevil

- a. Entomopathogenic nematodes have the greatest potential against black vine weevil during mid-spring, when most of the population is in susceptible, larger larval lifestages. However, the window for application and evaluation is usually short, especially at northern latitudes, where soil temperatures routinely remain below the thermal activity threshold for most commercial strains of entomopathogenic nematodes (15° C) until late spring. Weevils begin to pupate and emerge as adults when soils are at similar temperatures. Applications should be made when before adult emergence begins.
- b. The time frame for application of entomopathogenic nematodes against late instar root weevil larvae may be extended in strawberry beds by using mulches. Black plastic can raise soil temperature several degrees during early spring, but such a practice could also accelerate root weevil larval development.
- c. Alternatively, entomopathogenic nematodes can be applied in early fall when soil temperatures are still above 15° C, but most weevil larvae are still very small, less susceptible, and more difficult to detect. In faster developing crops such as caneberry, late summer applications may be further hindered by decreased irrigation after harvest.

#### 2. Cranberry girdler

- a. Entomopathogenic nematodes should be applied against cranberry girdler during mid-summer, when soil temperatures are warm and larvae are at least medium sized. Because moth emergence is often extended, the life stage distribution of girdler larvae is correspondingly mixed. Thus, an initial application at 3 weeks after peak adult emergence, as indicated by pheromone trap catches, should be followed by a second application 10 to 14 days later.

## VII. GENERAL APPLICATION METHODS

### A. Spot treatments, small infestations.

1. Sprinkling can to small areas
2. Subsurface injectors to individual plants. A fumigant injector was used to apply entomopathogenic nematodes to deep soil layers (> 10 cm) in grapes, but 50 separate injections of a 10 ml nematode suspension were required for each 1 x 0.5 m plot and treatments were not effective. Another type of subsurface injector was more efficient, but treatments still failed to effectively suppress grape root borer.
3. Small-scale water injector to temporary driplines. In a study conducted in western Washington red raspberry fields, entomopathogenic nematodes were applied through temporary overhead driplines using Mini-Dos® water-powered injector. Application time was rather slow (30 min/line), so the nematode suspension had to be frequently stirred by hand, but application density was fairly uniform.

### B. Large and whole-field infestations

1. Large scale injection pump to commercial driplines. Such a pump will evenly distribute the pathogen and also utilize a larger volume of nematodes in suspension which is easier to prepare and assess. However, commercial driplines frequently leak or are plugged, especially in the spring. Due to their high density and propensity to settle, nematodes may more likely be lost to small leaks compared to conventional pesticides.
2. Syringe to commercial driplines. Studies of dripline application to grapes featured injection of entomopathogenic nematodes by syringe, but until that method is more thoroughly tested.
3. Below ground T-Tape®. It has been demonstrated that entomopathogenic nematodes can successfully pass through below ground T-Tape in raised strawberry beds, but longer lines may be less effective.
4. Trickle irrigation. Nematodes may survive passage through the system, but distribution is far from uniform.
5. Set sprinkler system in cranberry. Studies have shown nematode densities to be fairly uniform after application through set sprinkler systems.

### C. Mulches to enhance nematode survival.

1. Compost mulches may enhance the survival of entomopathogenic nematodes, but could also introduce pestiferous nematodes.
2. Plastic mulches increase soil temperature, thus nematode survival and efficacy is enhanced, but so is the rate of pest development. Placement of black plastic immediately before application may be the best use. More research is needed.

## VIII. General Procedures

- A. Monitor hourly soil temperature at 10 cm depth beginning several days before application and continue throughout the duration of the experiment. In the Pacific Northwest during spring, soil temperatures may drop considerably after sunset, especially in tilled soil surrounding raspberry.
- B. Apply nematodes when soil temperatures exceed 15° C. For black vine weevil in the Pacific Northwest, ~50% of the population are pupae.
- C. Prepare nematode suspension and assay for concentration and viability. IJs can be easily counted in 0.3 ml aliquots using a stereomicroscope. At lower application rates ( $1-2 \times 10^9$  IJs/ha), the suspension need not be diluted, but higher rates may require dilution prior to counting. Count at least 6 aliquots per sample, and accept the average value if the standard deviation is  $< 1$ .
- D. Drench plots with water before and after application to stimulate nematode movement into the soil and wash them from foliage. Researchers have determined 1.25 cm water (2000 liters/ha) was appropriate for cranberry.
- E. Continue to measure nematode concentration and viability during application for uniformity in both space and time.
- F. Aerate or stir the suspension before and during application.
- G. Post-application Assessment (for persistence and uniformity of distribution)
  1. Soil Extraction. Techniques to isolate and quantify entomopathogenic nematodes directly from the soil are relatively complex and usually unnecessary to estimate nematode survival.
  2. Baited soil samples. Place alternate hosts such as wax moth (*Galleria mellonella*) larvae on soil sampled from treatment plots. Wax moth larvae are relatively inexpensive and readily available through the mail. Place 4-6 larvae per 30 g moistened soil in a sealed petri dish for 4 days at constant temperatures (20° C) to infection.
  3. Incubation. Maintain infected larval baits or cadavers 7 days in individual chambers at high relative humidity and constant temperatures.
  4. Buried baits. In some crops, baits can be buried in small screen cages for later recovery. Soils of cranberry bogs, however, are highly structured and strongly layered, so the burial of even very small cages would disrupt that structure and allow greater movements of entomopathogens through the soil. Strawberry fields are more frequently tilled, so buried baits may be more appropriate there.

# Control of Root Weevils in Nursery Crops with Entomopathogenic Nematodes

Ralph E. Berry

Dept. of Entomology, Oregon State University, Corvallis, OR 97331-2907

## Introduction

Control of root weevil larvae in nursery crops is notoriously difficult because of the cryptic nature of the larvae. Larvae feed on over 140 different species of host plants, but are particularly troublesome on azalea and rhododendron. Larvae feed from mid-summer into the fall, and again the following spring, when the most serious injury occurs. In mild climates, larvae also may feed during the winter. Larger larvae consume feeder roots and bark on larger roots in the spring, which results in reduced vigor of the plant and, in severe infestations, death of the plant.

Entomopathogenic nematodes have high potential for the control of root weevil larvae in several different nursery production systems. Several different nematode species have been shown to significantly reduce larval populations if applied properly. However, the use of nematodes on certain nursery plants with very low damage thresholds (such as rhododendron: 3 larvae/plant) may require more frequent applications at higher rates or their use may need to be integrated with chemical treatments.

The black vine weevil is the most common weevil species attacking nursery crops. The strawberry root weevil, while important in nursery crops, most commonly attacks small fruit crops, such as strawberry, cranberry and cane berries. Other weevil species, such as the woods weevil, rough strawberry weevil, clay-backed weevil, also may feed on nursery crops, but these species are not as abundant or damaging as the black vine weevil.

Black vine weevil larvae are highly susceptible to entomopathogenic nematodes. Infected larvae are killed within 48 hrs of attack and nematodes complete their life cycle within the host in about 14 days, depending on the temperature. Once the nematodes have completed their life cycle, the infective juvenile stage escapes into the soil in search of additional prey.

## The Nematodes

Many of the entomopathogenic nematode species that are available for insect control have been collected on ocean beaches around the world. Some species have been collected from infected insects, but for many species, the natural host is unknown. *Steinernema carpocapsae* is perhaps the most well known entomopathogenic nematode that has been commercialized. This nematode was first isolated from a codling moth larva. *S. riobrave* was collected in Texas and was originally isolated from a cotton bollworm larva. *S. glaseri* was isolated from a scarabaeid larva and *S. feltiae* was originally isolated from a bionid fly. There are many different strains of *Heterorhabditis*

*bacteriophora*, which was originally isolated from *Heliothis punctiger* in Brecon, Australia. Other strains of *H. bacteriophora* have been isolated from North Carolina and Utah. *H. megidis* was collected from a Japanese beetle larva in Ohio. The natural host of *H. marelatus* is unknown, but it has been identified from a sawfly larva collected along the Oregon coast.

Many nematode species have been collected from soil by baiting soil samples with waxworm larvae (*Galleria mellonella*) and dissecting *Galleria* to verify that nematodes are present. Many more nematode species are likely to be described as scientists sample different habitats around the world.

Most of the described nematode species belong in the genus *Steinernema*, a total of 24 species have been isolated, and four species have been commercialized. Of the *Heterorhabditis* species, only *H. bacteriophora* and *H. megidis* have been commercialized. It is likely that some additional species will be commercialized, but perhaps by small companies who can produce large enough quantities to supply local or regional biocontrol programs rather than enough to meet national needs.

It is important to select the right nematode species for a specific biocontrol program. For example, *S. carpocapsae* works best on Lepidoptera (pyralids), *S. feltiae* works best on sciarid flies, *S. scapterisci* controls mole crickets, *H. bacteriophora* and *H. megidis* both control scarab larvae, and *H. megidis*, *H. bacteriophora* and *H. marelatus* control root weevils. Steinernematids are less effective on root weevils than the heterorhabditids.

### **The Bacteria**

The bacteria associated with *Steinernema* spp. belong in the genus *Xenorhabdus*, whereas the bacteria found in *Heterorhabditis* spp. belong in the genus *Photorhabdus*. *Photorhabdus* bacteria are photoluminescent. This attribute is useful to determine the degree of infection of insect prey by nematodes. The intensity of the luminescence is greatest within the first 72 hrs after infection.

The symbiotic association between bacteria and nematodes benefits both organisms - one cannot live without the other. The bacteria that live inside the nematode are introduced into the insect prey by the nematode. Once inside the insect prey, the nematode inhibits the host's antibacterial defenses that would otherwise attack the invading bacteria. The bacteria produces a toxin that kills the insect prey and produces antibiotics that prevent the growth of other contaminating bacteria. The nematode feeds on the insect tissue and the developing bacteria and completes its life cycle. After the nematodes have completed their life cycles, the infective juveniles containing the symbiotic bacteria escape into the soil to search for additional insect prey.

The 3rd stage infective juvenile is the only nematode stage that can survive outside of the insect host. Infective juveniles disperse in the soil and search for additional prey. Once a suitable prey is located, the infective juveniles enter the host through natural body openings (spiracles, mouth, anus) or

through intersegmental membranes. Once inside, the bacteria are released and the insect host is killed within 48 hr. The nematodes complete their life cycle within the insect prey and infective juveniles are produced.

### **Nematode Life Cycle**

The life cycle of heterorhabditid nematodes is more complex than the life cycle of the steinernatids. In the steinernatids, both male and female infective juveniles enter the host. Mating occurs and the females lay eggs that develop into infective juveniles. In the heterorhabditid nematodes, the infective juveniles that enter the insect host are hermaphroditic females. Once these females have reached adulthood, they lay eggs that develop into males and females. When these nematodes reach adulthood, they mate and the females lay eggs that develop into the hermaphroditic female infective juveniles that escape from the insect host and disperse in the soil.

In general, under optimum conditions steinernatids require about a week to complete their life cycle, whereas heterorhabditids require about 2 weeks to complete a life cycle.



The mature females are often visible through the cuticle of the insect host. This photo shows the mature females of *H. marelatus* before the infective juveniles are produced.

### **Factors Determining Nematode Effectiveness**

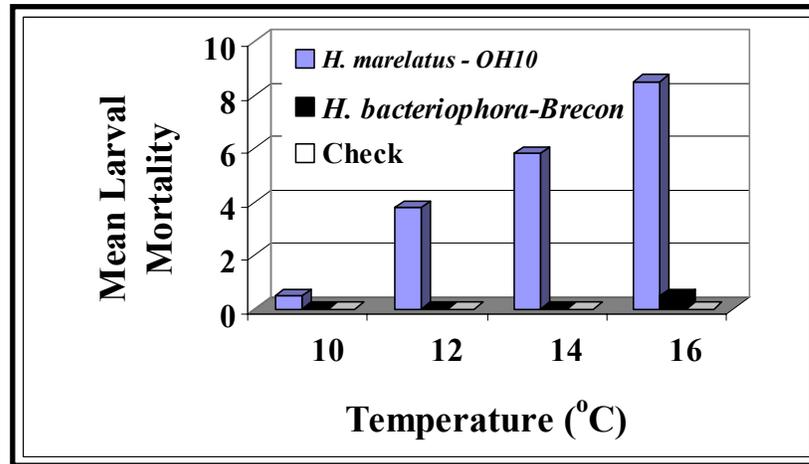
Many factors are involved in the success of entomopathogenic nematodes for biological control of insects. Each nematode species has specific attributes that contribute to its success on different insect hosts. Some nematode species are more virulent on some insect species than others even at comparable doses. The insect host also influences the susceptibility to nematode attack. For example, root weevils are often found deep in the soil and some species of nematodes are less effective in locating the prey. Environmental conditions, particularly soil temperature and moisture, are critical for the success of

entomopathogenic nematodes. Generally, nematodes are more effective in sandy soils than soils high in clay.

The virulence of the bacteria is perhaps the most important factor determining the effectiveness of entomopathogenic nematodes. Likewise, the immune system of the insect prey determines the success or failure of the nematode/bacteria attack. Some insects are less susceptible to some nematode species because they have the ability to “ward-off” the bacterial infection or are less susceptible to the toxin produced by the bacteria. Therefore, it is very important to select the “right” nematode species to use against the most susceptible insect prey.

In the laboratory, many nematode species can attack a large number of different insect hosts because they are often confined in time and space. As experiments move away from the laboratory, nematode host-searching behavior becomes more important. For example, some nematode species display an “ambusher” behavior to contact their prey (e.g., *S. carpocapsae*). These species tend to be associated with the upper levels of the soil and seldom go very deep in search of prey. Other nematode species display a “cruiser” behavior and actively search for prey deeper in the soil (e.g., *H. megidis*, *H. marelatus*). Nematodes that display the “cruiser” behavior are more effective against root weevil larvae than nematodes that display “ambusher” behavior. Other factors, such as host recognition, host defenses against attack and the host immune system play an increasingly important role under natural (field) conditions. The interplay of all of these factors is important for the successful biocontrol of insects with entomopathogenic nematodes.

Temperature is a critical factor for the success of entomopathogenic nematodes for biological control of insects. Some species of nematodes and their symbiotic bacteria are cool-temperature adapted, which may provide opportunities to control insect pests earlier in the spring. Applying nematodes in March or April in the Northern Hemisphere provides control of black vine weevil larvae and delaying applications until April has the added advantage of controlling larvae, pupae, and teneral adults, but it is disadvantageous because much of the plant damage has already occurred. Utilization of cool-temperature active nematodes, such as *H. marelatus* or *H. megidis*, may permit applications earlier in the spring when soil temperatures are lower than 14° C. We are especially interested in examining the use of *H. marelatus*, a cool-temperature species that was identified from the Oregon coast, for control of root weevils in the spring when soil temperatures are low.



*H. marelatus* controls strawberry root weevil larvae even at temperatures as low as 10°C. Control of weevil larvae increases as temperatures increase. Control of weevil larvae with *H. bacteriophora* is low even at 16°C, presumably because *H. bacteriophora* (Brecon strain) was originally collected from Australia and is adapted to warmer temperatures than *H. marelatus*, which was collected from the Oregon coast.

### Root Weevil Control

The use of beneficial nematodes against root weevil larvae is a prophylactic treatment rather than a curative treatment because it is very difficult to estimate the presence of larvae in the plant root system without destroying the plants.

Soil temperature and soil moisture are perhaps the two most important environmental factors for the success of beneficial nematodes for biological control of root weevil larvae.

Timing nematode applications is very important to coincide with the most susceptible stages of the weevil larvae. Applications in the late fall (late September - October) before soil temperatures begin to decline may be the best time to apply nematodes. However, nematodes are less effective against tiny larvae so applications must be timed carefully. Spring applications (late March - April) have the added advantage of controlling larvae, pupae, and teneral adults.

### Application Methods

The first step is to determine the surface area of the soil to be treated. Use the formula:  $\pi r^2$  to determine the surface area in in<sup>2</sup> or cm<sup>2</sup>. Measure the diameter of the container in inches or cm, if you measure in inches, convert to cm<sup>2</sup> by multiplying in<sup>2</sup> by 6.4516 (6.4516 is the conversion factor to convert in<sup>2</sup> to cm<sup>2</sup>). Once the surface area has been determined, calculate the number of IJs needed for a rate of 25 or 50 IJs per cm<sup>2</sup>.

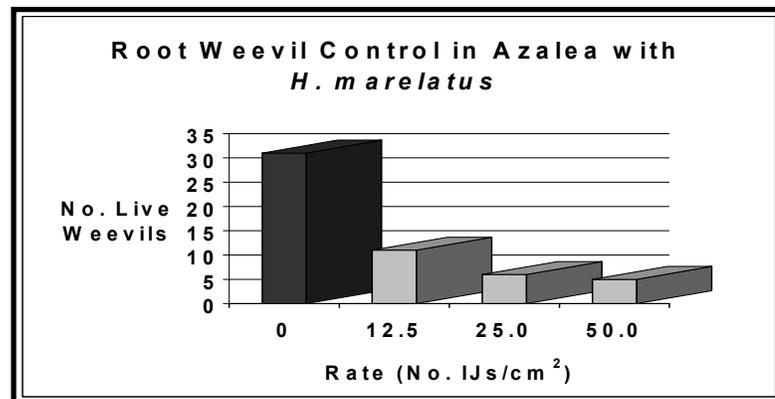
Nematodes can be applied by drenching or spraying the soil surface. Deliver the nematodes in 1 ml water per  $\text{cm}^2$  surface area. e.g., for a gallon-sized container (about  $182 \text{ cm}^2$ ) use 182 ml of water.

For rooted plants growing in the field, use the same procedures as above to calculate the surface area beneath the plant to be treated. Apply the nematodes as a drench or spray under the plant to the “drip line”. Apply in 1 ml of water per  $\text{cm}^2$  or in 1 liter of water per  $\text{m}^2$ .

In addition to drenching or spraying the nematodes on the soil surface, the nematode suspension may be injected into the soil at different locations around the plant, or containers may be dipped in a suspension of nematodes, or nematodes may be applied through overhead sprinklers or through drip irrigation systems.

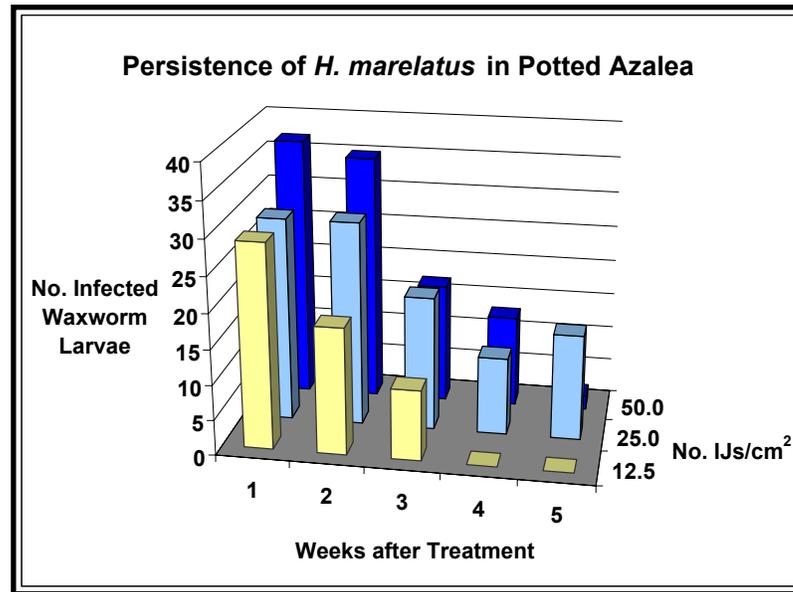
It is important to pre-moisten the soil surface before application and to follow treatment with additional irrigation. The soil moisture should be maintained for about 2 weeks after treatment to maximize control.

If possible, containers can be treated in groups. Plants on pallets can be treated as a group or plants arranged on a greenhouse bench can be treated at the same time. If individuals are interested in determining effectiveness of the nematode treatments, some containers can be removed from the treated areas and destructively sampled after about 2 weeks to recover infected larvae. For the heterorhabditids, the larvae will be red if they are infected with nematodes.



In most situations, rates of  $25.0 \text{ IJs/cm}^2$  (equivalent to 1 billion IJs per acre) or  $50.0 \text{ IJs/cm}^2$  (equivalent to 2 billion IJs per acre) are adequate to control root weevil larvae. Studies show that the efficacy of the nematodes increases as the rate increases, but there is little difference between 25 and  $50 \text{ IJs/cm}^2$ . It should be noted that nematode species other than *H. marelatus* may require higher rates to be effective against root weevil larvae.

Nematodes persist in the soil for up to 5 weeks after treatment, but their effectiveness declines during this period. Nematodes enter their host, release the symbiotic bacteria, and kill the host within 48 hours after treatment so the length of time the nematode persists in the container is not critical to their success.



The degree of control offered by nematodes is high enough that recycling of the nematodes to kill additional prey is seldom necessary. However, because they are highly efficacious, they need to be re-applied each year (one treatment in the fall or spring for root weevils because they only have one generation each year).

Drenching the nematodes directly on the soil surface is perhaps a better method of application than spraying the nematodes over the foliage. Some of the nematodes land on the foliage and do not reach the soil surface or they may land outside of the target area or desiccate on the foliage. Effectiveness of nematodes against root weevil will depend on each production system and the effectiveness of the application method used.

### Conclusions and Recommendations

Infective juveniles applied at 25 or 50 IJs per cm<sup>2</sup> provide excellent control of root weevil larvae. Our results show that applying the nematodes either as a drench or sprayed over the foliage effectively delivers the IJs to the target area. Even though we have not evaluated other methods of application, injecting the nematodes into containers or dipping the containers in a suspension of nematodes would also be effective methods of delivering the nematodes to the target area. Each nursery will have different situations and methods of application should be tailored to each nursery's needs.

Perhaps the easiest method of application is through overhead sprinkler irrigation or drip irrigation systems. However, the equipment needed to accurately deliver the correct rate to the entire area needs to be carefully calibrated. Whatever application method is used, it is important to pre-wet the soil and irrigate after treatment to keep the soil moist.

The cost of using beneficial nematodes in containers or for rooted plants is very reasonable if the nematodes can be delivered precisely to the root areas of plants. At a rate of 50 IJs per cm<sup>2</sup> in gallon-sized containers, the cost for material is only about \$0.004 per container. For a rooted plant, the cost of 50 IJs per cm<sup>2</sup> would be about \$0.02 to treat a plant 24 inches in diameter.

Nematodes applied in the fall or spring have been shown to provide control of root weevil larvae. In the fall, treatment should be delayed until late September or early October to increase efficacy since nematodes are less effective against small larvae. In the spring, nematodes control larvae, pupae and teneral adults when applied in late March or April after soil temperatures begin to increase and before weevil development is completed. It is important to apply the nematodes when the soil temperature and moisture are favorable as discussed earlier.

Heterorhabditid nematode species have been shown to be more effective than the steinernematid nematodes perhaps because of their different searching behaviors - most heterorhabditid species have a "cruiser" behavior whereas most steinernematids have an "ambusher" behavior. Nematodes with a cruiser behavior often go deeper in the soil to locate the root weevil larvae. Two heterorhabditid species, *H. marelatus* and *H. megidis*, have been shown to be effective against root weevils when soil temperatures are 50 to 55°F. *H. bacteriophora* is effective when soil temperatures reach at least 60°F.

## Useful Conversions and Examples

<p>1 inch = 2.54 cm</p> <p>1 sq in = 6.4516 sq cm</p> <p>1 Gallon = 3.78 Liters</p> <p>1 Liter = .26 Gallons</p> <p>128 fluid ounces per gallon</p> <p>1 fl. ounce = 29.6 cc (29.6 ml)</p> <p>1 Liter = 1000 ml (1000 cc)</p>	<p>1 Billion Infective Juveniles (ijs) per acre is equal to:</p> <p style="padding-left: 20px;">~ 22,957 ijs/ft<sup>2</sup>, or ~ 159 ijs/in<sup>2</sup></p> <p>Metrically, the equivalent rate is</p> <p style="padding-left: 20px;">2.47 Billion ijs per Hectare, or ~ 247,000 ijs/m<sup>2</sup>, or ~ 24.7 ijs/cm<sup>2</sup></p>
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Nematodes are applied based on the surface area to be treated, **NOT VOLUME**, of soil. For square or rectangular containers, surface area is found by multiplying the length by the width of the area to be treated. For round pots, surface area is determined by the equation:  $\pi r^2$ , where  $\pi$  is equal to (3.1415) and r is the radius of the pot (not the diameter).

Here are some examples:

1. Let's say you have a bench of 4" square pots that you want to treat. How many can you treat at a rate of 1 Billion ijs per acre if the nematodes are supplied in bags of 50 million per bag?
  - 4" x 4" = 16 square inches per pot
  - 16 in<sup>2</sup>/pot x 159 ijs/in<sup>2</sup> = 2,544 ijs/pot
  - 50,000,000 ijs/bag ÷ (2,544 ijs/pot) = **19,654 4" square pots/bag**
  
2. If we want to treat a house full of plants in 6" round pots at a rate of 2 Billion ijs per acre, how many ijs per pot will we need, and how many pots can we treat with a pack of 25 million ijs?
  - 3.1415 x (3")<sup>2</sup> = 28.3 square inches/pot
  - 28.3 in<sup>2</sup> x (2 x 159 ijs/in<sup>2</sup>) = 8,999 ijs/pot
  - 25,000,000 ijs/pack ÷ (8,999 ijs/pot) = **2,778 6" round pots/pack**
  
3. If we want to treat the round pots in example #2 with a 3 ounce drench per pot, what volume of water do we need to treat all the pots?
  - 2,778 pots x 3 ozs = 8,334 ozs
  - 8,334 ozs ÷ 128 ozs/gal = **65.1 gal**
  
4. Now we need to treat our blueberries with nematodes. Let's figure we have 100' rows and we will treat a 1' area (6" on each side of the plant). How many ijs will we need to treat 25 rows if we apply them at a rate of 1.5 Billion per acre?
  - 100'/row x 1'/band x 25 rows = 2,500 ft<sup>2</sup>
  - (22,957 ijs/ft<sup>2</sup> x 1.5) x 2,500 ft<sup>2</sup> = **86.1 million ijs**

Okay, now for some real calculations!

5. Suppose we have 15,000 6" round pots to be treated at a rate of 2 billion nematodes per acre. All of the pots are on a microirrigation system, and we have an injector pump that is capable of injecting 12 gallons per hour. 1) How many nematodes do we need per pot? 2) How many nematodes do we need total? 3) How long will it take to inject all of the nematodes if they are mixed in 10 gallons of cool water?
  1. Area of each pot =  $\pi r^2$ , so,  $(3.1415) \times 3^2 = 28.27 \text{ in}^2/\text{pot}$   
 $28.27 \text{ in}^2/\text{pot} \times (159 \text{ ijs/in}^2 \times 2) = \mathbf{8,990 \text{ ijs/pot}}$
  2.  $8,990 \text{ ijs/pot} \times 15,000 \text{ pots} = \mathbf{134,850,000 \text{ ijs}}$
  3. Max. pump output is  $(12 \text{ gallons/hour}) \times (1 \text{ hour}/60 \text{ minutes}) = 0.2 \text{ gpm}$   
 $(10 \text{ gallons}) \div (0.2 \text{ gpm}) = \mathbf{50 \text{ minutes}}$

Chemicals found to affect nematode efficacy when exposed. These should be applied with care when used in conjunction with nematodes. Compiled from D. Shetlar, 1999 and K. Smith, 1999.

## CHEMICAL USE PATTERNS WITH NEMATODES

### Tank Mix

<u>COMPOUND</u>	<u>TRADE NAME</u>	<u>COMPOUND</u>	<u>TRADE NAME</u>
Acephate	Orthene	Fenoxycarb	Logic
Azadirachtin*	Azatin, Neem*	Fertilizers	Various
<i>Bacillus thuringiensis</i> (Bt)	M-One,	Fipronil*	Chipco Choice*
	Dipel	Fosethyl-Al	Aliette
Benomyl	Benelate	Glyphosate	Roundup
Bifenthrin	Talstar	Insecticidal Soap*	Various
Bromine-chlorine	Agribrom	Iprodione	Chipco 26019
Carbaryl*	Sevin*	Isofenphos	Oftanol
Carbofuran*	Furadan*	Kinoprene	Enstar
Chlorothalonil	Daconil	Metalaxyl	Subdue
Chlorthal dimethyl	Dacthal	Methidathion	Supracide
Copper Hydroxide	Kocide	Methomyl*	Lannate*
Cyfluthrin	Tempo	Methoprene	Apex
Cythion	Malathion	Oryzalin	Surflan
Diazinon	Knox-out	Oxamyl*	Vydate*
Dienochlor	Pentac	Oxazoidinedione	Ornalin
Diflubenzurion	Dimilin	Pentachloronitrobenzene	Terrachlor
Endosulfan	Thiodan	Thiophanate-methyl	Zyban
Esfenvalerate	Asana	Triademefon	Bayleton
Etridiazole	Terrazole	Trichlorfon*	Dylox, Proxol

#### 1-Week Separation

<u>COMPOUND</u>	<u>TRADE NAME</u>
Bendiocarb	Turcam, Ficam
Chlorpyrifos	Dursban
Anilazine	Dyrene
Dimethyl benzyl ammonium chloride	Physan 20
Fenarimol	Rubigan
Mercurous chloride	Calo-Clor
2,4-D	Various
Triclopyr	Turflon, Confront

#### 2-Week Separation

<u>COMPOUND</u>	<u>TRADE NAME</u>
Ethoprop	Mocap
Fenamiphos	Nemacur
Isazophos	Triumph

\* Use pattern not well-established. Monitor closely.

# Useful Websites

## NEMATODES FOR SALE

<http://www.allorganics.com/> Have nematodes, but don't list species.  
[www.asajunglab.com](http://www.asajunglab.com) *S. carpocapsae/S. feltiae* blend; not much technical info.  
<http://www.biconet.com/flies/nematodes.html> *S. carpocapsae*; not much technical info.  
<http://www.biologicco.com/> Have *S. carpocapsae*, *S. feltiae*, *H. bacteriophora*.  
[www.biopest.com](http://www.biopest.com) Have "nematodes," but don't list species.  
<http://www.buglogical.com/> Have *Steinernema* and *Heterorhabditis*, but don't list species.  
<http://www.bugstore.com/> Have *Steinernema* and *Heterorhabditis*, but don't list species.  
[www.down-to-earth.com](http://www.down-to-earth.com) Have "nematodes", but don't list which ones.  
<http://www.goodbug.com/nema.html> Have *S. carpocapsae*, *H. bacteriophora*, no info on rates.  
<http://www.goodbug-shop.com/> Have *Heterorhabditis* and *Steinernema*, several species.  
<http://www.hydro-gardens.com/> Have *Steinernema sp.* and *Heterorhabditis sp.*  
[www.koppert.com](http://www.koppert.com) Have *S. carpocapsae*, *S. feltiae*, *H. megidis*.  
[www.mellingers.com](http://www.mellingers.com) *S. carpocapsae* only.  
[www.mycotech.com](http://www.mycotech.com) Have *S. feltiae*, *S. carpocapsae*, *H. megidis*.  
<http://www.natural-insect-control.com/pg19.html> Have *S. carpocapsae*, *S. feltiae*, *H. megidis*.  
[www.territorial-seed.com](http://www.territorial-seed.com) *Steinernema/Heterorhabditis* blend? No species listed for either.  
[www.wormsway.com](http://www.wormsway.com) "Good nematodes", no other information.

## NEMATODE INFO

<http://www2.oardc.ohio-state.edu/nematodes/> Good, all-around site.  
<http://www.res.bbsrc.ac.uk/entnem/>  
<http://pppweb.clemson.edu/NematodeSites.html>  
<http://www-rci.rutgers.edu/~nematode/>  
<http://ianrwww.unl.edu/ianr/plntpath/nematode/nemabib.htm>  
<http://csssrvr.entnem.ufl.edu/CTLWeb/nem.htm>  
<http://gnv.ifas.ufl.edu/~kbn/kbnstein.htm>  
[http://www.spg.wau.nl/nema/ident\\_lit.html](http://www.spg.wau.nl/nema/ident_lit.html)  
<http://www.ianr.unl.edu/pubs/NebFacts/NF182.HTM>

## OTHER HELPFUL WEB PAGES

OSU Integrated Plant Protection Center (IPPC)

<http://ippc.orst.edu/>

OSU Extension Communications

<http://www.wagcomm.ads.orst.edu>

NWREC Homepage

<http://www.orst.edu/Dept/NWREC/>

NWREC Berry & Grape Information Network

<http://www.orst.edu/dept/infonet/>

Oregon Department of Agriculture

<http://www.oda.state.or.us/oda.html>

Oregon Agriculture Statistics Service

<http://www.oda.state.or.us/oass/oass.html>