



Biology of Entomopathogenic Nematodes (EPN)

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Stylet

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What are nematodes: Nematodes are often referred to as round, eel or threadworms because of their cylindrical and elongated bodies. These non-segmented, invertebrate animals range in size from 0.1 mm to several metres in length. Over 28,000 species have been recognized worldwide and over 16,000 are parasitic. It has been estimated that there are approximately 1,000,000 different nematode species in the world.

Beneficial or Entomopathogenic Nematodes (EPNs): The term entomopathogenic comes from the Greek word entomon referring to insect, and pathogenic, which means producing disease. EPNs of the families called Steinernematidae and Heterorhabditidae are lethal pathogens of insects. All

EPNs are symbiotically associated with bacteria located in their intestinal tract.

Host search behaviour & EPN strategies: This can be divided into two broad categories: ambushing and cruising. Ambusher species such as *Steinernema carpocapsae*

Top. EPN application with a backpack sprayer. **Adjacent.** Nematodes parasitic on plants obtain food by sucking juices from them. Feeding is accomplished through a hollow, needlelike mouthpart called a stylet. The nematode pushes the stylet into plant cells and injects a liquid containing enzymes, which digest plant cell contents. The liquefied contents are then sucked back into the nematode's digestive tract through the stylet.

tend to remain stationary; they search by standing on their tail and elevating most of their bodies. This sit-and-wait approach to finding hosts serves as a mechanism for host attachment. Ambushing is clearly an adapted behaviour in the top two inches of non-compacted soil, as it is not possible to do this function effectively deeper in the soil. Cruiser nematodes such as *Heterorhabditis bacteriophora* and *S. glaseri* tend to be highly mobile searching in comparatively large areas for hosts. They are highly responsive to host-released volatiles like CO₂ that they use to orient toward insects. Cruiser species are found distributed throughout the soil profile as would be predicted from their search behaviour. Cruiser nematodes are best adapted to parasitize sedentary, below ground hosts such as white grubs. The cruisers tendency is to move downward about eight to ten inches and horizontally up to 10 feet to seek their host.

Major turf grass grubs & identification:

Some of the most important white grubs in turf grass are: European chafer (*Rhizotrogus majalis*), June beetle (*Phyllophaga spp.*), Japanese beetle (*Popillia japonica*), Asiatic garden beetle (*Maldera castanea*) and black turf grass *Ataenius* (*Ataenius spretulus*). The first three species are the most common pests in Ontario and throughout most of Canada. Correct identification of white grub species is important in determining management strategies and timing of controls.

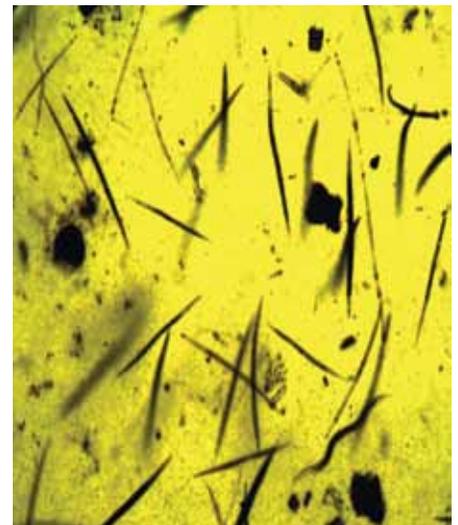
To identify different species we should observe the grub's raster (arrangement of bristles and hairs on the underside tip of the abdomen). A 10x hand lens is needed to see this pattern on most mature white grubs.

Ordering, Receiving & Storage

Ambusher or cruiser, which one to order:

According to the type of pests, we can determine which category would be the better choice for application. Generally, to control soil-surface pests, ambushers are more effective. Conversely, to control pests in deeper areas of soil, the cruiser nematode will produce the highest efficacy.

Labeling and necessary information: For



assurance of EPN quality and their efficacy, all the nematode packages should have a label with the necessary information including: nematode species, quantity, application rate and the expiration date.

Viability: There are different methods for checking EPN's viability. First of all, check the attached label and consider the expiry date. If nematodes are on a sponge, make sure it was shipped with icepacks overnight and refrigerated upon receiving. Fresh nematodes will have a slight earthy smell and appear light gray, beige or pinkish (depending on strain). Dying or dead nematodes will have a strong putrid odour. If nematodes are in powder, the same indication as above applies, but be careful that the plastic bag is clean and free of any fungus infection symptoms.

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Monitoring for freshness & activity assurance:

In case of a sponge carrier, after opening a package, extract a tiny sample of nematode paste by using a small utensil and place it on flat clear surface (solo cup lid) then mix 2 ml of tap water with nematode paste and in case of a powder carrier, mix and dissolve approximately 1 g to 20 ml of tap water. Separate 2 ml on a flat surface. In both cases, look at the suspension by use of magnifier equipment. The fresh nematodes are active and their

Above. Nematodes under the microscope. On the left they are alive; on the right they are dead.

bodies wiggling under at least a 10x hand lens or a microscope.

Desired Conditions Before Applying EPN

Moisture: Sufficient soil moisture plays a main role in the infective juvenile's (IJ) efficacy and survival. EPNs use the water channels like roads to reach their hosts. Therefore the soil needs to be moist down below the level of the grubs. Keeping the soil wet for 2-3 days after an application can provide an ideal situation for EPN efficacy.

Texture: the capacity of different soil textures in holding oxygen is an important factor in the IJ's survival. For example: a field trial in different soil textures confirmed that the lowest survival for EPNs was recorded in the clay soil (compared to sand, sandy loam and clay loam). This lower survival rate is probably related to the lower oxygen level because of small pores in clay soils. Note: if the soil is saturated from heavy rains or lack of drainage, the nematodes could die from lack of oxygen.

A soil sample infested with European chafer.



Temperature: The effect of temperature on survival varies with nematode species and strains. Nematode species isolated from temperate regions tend to be more tolerant of low temperature than species isolated from tropical or subtropical regions. Soil temperature determines the activity and efficacy of EPNs. If is too cold, they are inactive and therefore will not seek the hosts. Conversely, if the soil is too warm, they will use up their energy source too quickly.

U.V.: Nematodes will die from ultraviolet light. From a practical viewpoint, the application of EPNs is recommended in early morning, late afternoon or on cloudy days to minimize detrimental effects of desiccation, ultraviolet light and extreme temperatures.

Application Time

Fall & spring: Fall application is a crucial factor to control pest population and their damage. Application from August to the end of September (depending on the weather and soil temperature) when the young instar of European chafer and Japanese beetle larvae are hatched from their eggs would be the an ideal time for EPN application. The 1st and 2nd year grubs of June beetle can also be controlled at this time. Fall applications will decrease grub populations under threshold levels,

which means preventing both fall and spring damage.

Grubs in Ontario generally overwinter as larvae. With increasing weather and soil temperatures, overwintering larvae will move from the deeper soil depth to the upper layer. When soil temperatures rise above 10° C (late April to early May in Ontario), this would be the time for EPN application. The EPN spring application (even against the last instar of pest larvae, which have tougher bodies) can reduce the total number of grubs by reducing the number of fertilized adults in the next months. Note: Spring application will be necessary only if grubs are found in the soil. This application indicates necessity for fall application.

Method of Application

Home gardeners & landscapers: First step is to pre-water the area being treated thoroughly 5-8 cm deep. If EPNs are on sponges, remove sponge from sealed bag and rinse both sponge and bag in a minimum of 4 litres of water to make a concentrate. Stir the concentrate vigorously to suspend nematodes equally as they are heavier than water. Use a hose-end sprayer or pump sprayer to apply immediately (100 gal/2000 ft²). In powder carriers, the nematodes should be diluted in a large amount of water (a minimum of 25 gal) and apply immediately (2.25 gal/ 100ft²).

Turfgrass: For larger areas, nematodes can be applied by using conventional agitating spraying systems such as pump, back-pack, truck sprayers, an overhead/misting sprayer system or soil injecting sprayer. Nematodes can be sprayed using a common type nozzle with an opening ranging from 50 microns to 1 mm. It is recommended to remove the finer screens. Recently, a soil injecting nematode applicator was developed for turfgrass which potentially could increase nematode efficacy and may decrease the cost for application.

Post Application & Monitoring For Controlled Results

Expected time for pest control: It is theoretically possible for EPNs to kill their host after 48-72 hours, but because of natural barriers in the soil, it is highly unlikely this occurs in the field.

Reapplication: The same as all other pesticides, EPN application will not obtain 100% mortality in the grub's population. A percent of beneficial nematodes can survive during the winter in the soil, but to reach a higher level of pest control, reapplication will be necessary.

Conclusion

- Know the pest insect and timing of its life cycle.
- Know the temperature of the soil.
- Know that the larvae are present.
- The soil must be wet to below the level of the grubs.
- Apply in low UV times.
- Water well after application to ensure the nematodes are past the surface layer and down to the root level of the grass.
- Keep the soil watered for 3-4 days after application.
- A spring infestation indicates a fall application must be done.

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