Entomopathogenic nematodes

Entomopathogenic nematodes (ENs) can be used beneficially to attack insect pests. The nematodes are attracted to the insect larva by its carbon dioxide exhalations, travelling towards it in the thin film of moisture that surrounds the particles of the medium. They enter through its body openings, at the same time releasing highly specialised symbiotic bacteria which multiply within the larva. The bacteria, harmless to humans and other animals, serve as a food source for the nematodes.

After approximately 14 days the nematodes have reproduced inside the victim and the larva is dead. The dead larva ruptures and releases up to 100 000 times the number of ENs that had originally infected it; these then go in search of new prey.

Plate 80: Infective juvenile nematodes. Note the transparent protective sheath.

ENs available and their target pests

Nematode species	Target pests
Heterorhabditis bacteriophora	• black vine weevil
Heterorhabditis zealandica	• Argentine stem weevil • African black beetle • black-headed cockchafer • Argentine scarab • bill bug weevil
*Steinernema carpocapsae**	• banana borer weevil • cutworm • armyworm • house termites • cat flea
Steinernema feltiae	• mushroom fly • fungus gnat
*Beddingia siricidicola**	• sirex wasp

*Not covered in detail within this book

Applying nematodes

Most ENs are formulated in a cellulose mixture, and are supplied at a stage where they are ready to infect insect larvae. They are sold in large and small packs; the large pack contains 50 million nematodes and the small pack 25 million. During the formulation process they are dehydrated to a stage where their metabolism is reduced to only 1% of its normal rate.

Before being applied, the nematodes need to be rehydrated in water for approximately 15 minutes. Then they need to be applied to a pre-moistened area, close to the pest, with adequate water to allow the nematodes to disperse into the medium.

ENs will be most effective if used as soon as possible after they are received. They can be stored and still work effectively, but their viability will diminish over time. ENs vary in storage requirements, so refer to the supplier's instructions. Some types store at room temperature whereas others require refrigeration.

Before application

Remove all filters smaller than 30 mesh (500 micron) from the application equipment. Pre-irrigate the area to be treated, or ensure that the area is already sufficiently damp to allow nematodes to move freely through the medium. Check previous chemical application history to ensure that only compatible chemicals have been applied to the area. Refer to your nematode supplier for the most recent information on chemical compatibilities. Ensure that the medium is within the temperature range of 12–30ºC.

Application rates

Application rates and timing are provided in the specific entries in this book, or are available from the supplier.

Cultural practices to aid nematode establishment

Apply ENs when ultraviolet (UV) levels are at their lowest, because UV light can have a detrimental effect on nematodes at application time. Subsequent irrigation will help wash any nematodes that have been caught in the leaf canopy back into the medium. Nematodes need to move around in the thin film of moisture present in the medium to seek out their prey.

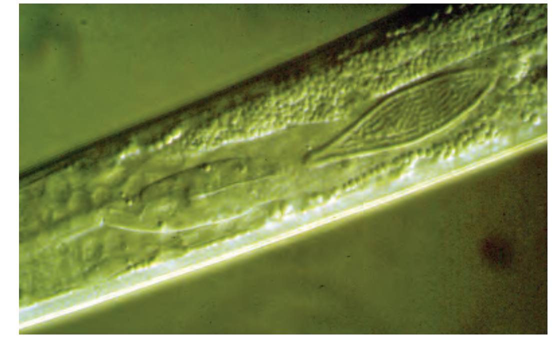

Plate 81: Cross-section of nematode, highly magnified to reveal bacterial sacs

Chemical use

Infective juveniles are compatible with most, but not all, agricultural chemicals under field conditions. *Heterorhabditis bacteriophora* is the most sensitive among entomopathogenic nematodes to physical stress, yet even this species tolerates many fungicides, herbicides, insecticides and nematicides.

Moreover, many of the chemicals noted as toxic had a transient effect only; the nematodes recovered quickly when the exposure ended. Thus, tank mixes and simultaneous application of nematodes with most pesticides and fertilisers should be feasible, provided exposure is short. Refer to compatibility charts from the supplier for an indication of the effects of exposure to commonly used products.

Safety

Various tests against mice, rabbits and monkeys have shown that the ENs tested are harmless when fed, injected or inhaled. They are also harmless to earthworms and other non-insect organisms, including plants, and they are non-polluting. Environmental protection agencies in the USA and many other countries including Australia have exempted ENs from registration due to their low toxicity.

However, as with a variety of materials, from pollen and flour to various insects and plants, there is always the possibility that some individuals may develop allergies to ENs; so it is wise to take steps to prevent the possible inhalation of EN sprays and to protect the skin from contamination.

Heterorhabditis bacteriophora
Entomopathogenic nematode

Target pest

Black vine weevil ***Otiorhynchus sulcatus***

This pest is an introduced species from Europe that has adapted well in the cooler areas of Australia. Larvae are often spread when potted plants are moved.

Black vine weevil undergoes complete metamorphosis (egg, larva, pupa, adult), usually with only one generation each year. The female can lay up to 1000 eggs over several months. The eggs are white initially, before turning to brown; they are roughly spherical and 0.7 mm in diameter. They are usually deposited in the soil or potting medium near the base of the plant. They hatch into larvae after 10–25 days.

The larvae are white, curved, legless and about 10 mm long when fully grown. They have orange to brown heads. The larvae may appear whitish pink when very young. The larvae live in the ground for about 6–9 months, and most of them pupate in late winter or spring. The pupa is about 8 mm long, with the wing cases free; it remains in this state for approximately 20 days. Adult vine weevils are about 10–12 mm long and are brown to black with faint yellow spots. They are flightless and have the typical elongated snout of a weevil.

It is mainly the larvae that cause damage to plants. They feed voraciously on the root system and may be found from near the surface to as much as 40 mm down in the soil near the roots. Cyclamen corms may have hollows chewed into them by larvae, and various woody plants may be ringbarked at the soil surface interface.

Adult weevils produce telltale notches around the edges of leaves and flowers. They are most active at night, so are seldom associated with the damage. Adults may also chew fruit stalks or grapes. On rhododendrons and azaleas the larvae may girdle the plant by chewing bark from the lower stems.

Suitable crops/environments

The list of ornamentals affected by the black vine weevil is quite extensive, with at least 300 species known to be affected. These include cyclamen, heuchera, maidenhair fern, polyanthus, fuchsia, strawberry, raspberry, rhododendron, azalea, yews, conifers, walnuts, begonias, impatiens and geraniums.

The nematodes can be applied as a drench or spray drench with water to the growing media in potted crops, or to garden beds in the landscape situation. Apply when soil temperatures have increased above 12ºC.

Proper application and use of nematodes varies with different crops and production systems, but equipment normally used for spraying pesticides will be suitable for most applications. Optimum temperatures are between 15˚C and

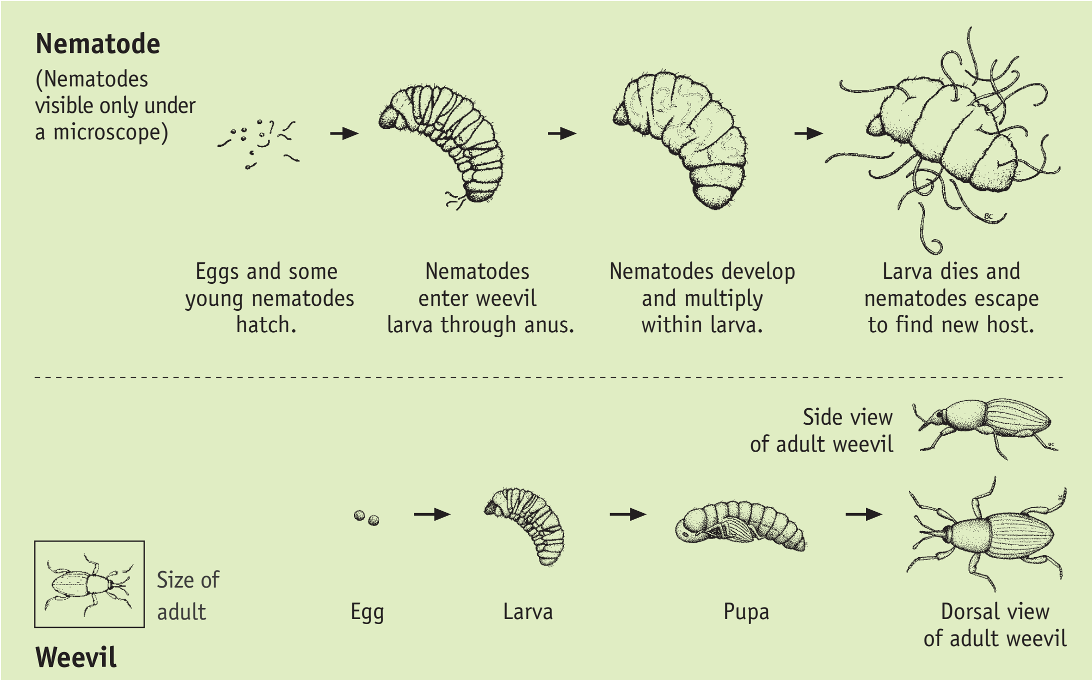

Figure 16: Life cycles of nematode and weevil

28°C. At temperatures over 30°C or below 12°C the nematodes become inactive. However, the protected growing environment of a nursery rarely exceeds these limits within the growing medium.

Application

Recommended application rates

The number of nematodes applied per square metre is the important factor. The amount of water used to apply them will be a function of the method of application. In situations where it is difficult to apply the recommended dose to the target area, such as to individual pots, it is advisable to add twice as much water as recommended and then apply twice the dose. Any wastage between target areas is thereby reduced.

Preventive treatment: The recommended density for preventive treatment is 50 million per 200 m^2 of growing area. This is equivalent to a rate of **250 000 per m^2**, or 50 million per 5000 × 200 mm pots, since there are 25 × 200 mm pots per m^2. This rate will be effective only if the pest is present in small numbers.

The first opportunity to apply nematodes targeting black vine weevil occurs in late March and is over by early May. There are variations in the best timing for application due to seasonal weather variations or the geographical location of the site. The first opportunity is referred to as preventive, because the larvae are very small at this stage and physical monitoring can often give a misleading indication of the real situation. It is therefore better to apply this treatment as a routine part of an annual control strategy.

Curative treatment: For curative treatment, use 50 million per 100 m^2 of growing area. This is equivalent to a rate of 500 000 per m^2, or 50 million per 2500 × 200 mm pots. Add one large pack to 50 litres of water in order to distribute an individual dose of 20 mL of solution to 2500 pots (e.g. 20 mL × 2500 pots = 50 L).

From early September to early October is the second window of opportunity for controlling black vine weevil. Leading up to this period the larvae will be quite noticeable, so monitoring techniques can be useful for identifying the presence of the pest.

The factor that determines the timing of the second and final treatment is temperature. Soil temperatures must be above 12°C. Once pupation has occurred (late September or mid-October) the pupae (about 8 mm long with the wing cases free) are still relatively sensitive to the nematodes, but the crop damage has been done. This 'window' is shorter than the first; hence the importance of using both opportunities to effect an overall control strategy.

After application

Maintain adequate moisture levels for the next few weeks. Randomly check the roots of susceptible plants for larvae and reapply nematodes to hot spots. Inspect plants regularly during the spring and summer for telltale notching of the leaves.

In hot weather, check the root system of plants that appear healthy but look stressed when subjected to slight drought, or continue to wilt when well irrigated.

Adults can be monitored from December onwards. Check under the rims of pots for adults hiding during the day, or place a trap board (a sheet of wood or iron which they will use as a shelter during the day) to attract adults, which can then be physically removed.

Heterorhabditis zealandica
Entomopathogenic nematode

Target pests

- African black beetle *Heteronychus arator*
- Argentine stem weevil *Listronotus bonariensis*
- Black-headed cockchafer *Aphodius tasmaniae*
- Argentine scarab *Cyclocephala signaticollis*
- Bill bug weevil *Sphenophorus brunipennis*

Heterorhabditis zealandica attacks a range of insect pests, but African black beetle in turf is the main target at present in Australia. The beetle is an introduced pest from South Africa and has left behind its natural predators and pathogens. It has been a pest of Australian horticulture, forestry and turf since the early 1900s. At present its distribution is coastal mainland Australia from Brisbane to Melbourne, as well as coastal South and Western Australia, although its potential distribution is believed to be from far north Queensland to southern Tasmania.

Plate 82: African black beetle adult

African black beetle has one generation per year. The eggs are typically laid in October and hatch into first-instar larvae in November, developing through the second instar and to third-instar stage by late January. The third-instar larvae are around 12–15 mm long, with a tan to yellow head and the characteristic curl shape, dark rear end and six legs of the scarab group.

The larvae need to feed well to develop fully through the pupal and adult stages of the life cycle. They eat plant roots, leaving the turf weak, easily damaged and prone to summer drought stress. Birds can also cause considerable damage while foraging for these grubs.

After the third-instar stage the larvae tunnel deeper to pupate (February). Adults emerge through February and March, and launch into their dispersal flight. Little is known about the distance of these flights.

When they land, the adult beetles burrow into the thatch and soil, where they continue feeding in a minor way, and they overwinter in a protected site. During this overwintering period the main population reaches sexual maturity.

As the weather warms in September and October the beetles emerge to roam on foot in the thatch and grass layer, searching for a mate. Once mating has occurred the impregnated female can carry the male's sperm until she is ready to fertilise and lay the eggs. Eggs are laid in the soil, just under the thatch layer, usually between late September and late October. The females produce about twelve eggs, almost all of which hatch successfully to larvae. A very wet spring, however, may reduce numbers by drowning.

Plate 83: Nematodes within an African black beetle larva

Suitable crops/environments

African black beetle prefers couch grasses but can also be found in kikuyu and cool-season turfs. Golf courses, playing fields and parks that contain these grasses are all potential candidates for nematode applications.

Application

Recommended application rates

Application rates depend on the specific pest being targeted. Nematodes are supplied as treatments for specific pests and areas: full hectare (10 000 m^2); half hectare (5000 m^2); bowling green pack (5000 m^2); and domestic pack (200 m^2).

Plate 84: Nematode-infested scarab beetle larvae, with a healthy larva in the centre for comparison

The amount of water used to apply the nematodes will depend on the method of application. A minimum of 500 litres per hectare should be used for broadacre applications, and domestic users should use a minimum of 20 litres per 100 m². The most important considerations are that there is sufficient water to allow the nematodes to seek out their prey, and that subsequent irrigation washes the nematodes off the leaves and into the soil.

Application method

All treatments are supplied as a one-off, but a follow-up application may be necessary 4–6 weeks after the initial application to account for overlapping generations of some species. African black beetle should require only a single treatment. Ensure all post-irrigation is completed within one hour of application. Refer to supplier for specific requirements for each pest.

Generic application rules

- Ensure spray equipment has been rinsed and all filters have been removed.
- Always apply nematodes at dusk. Do NOT apply at other times, even if the weather is overcast.
- Do not apply when the ambient temperature exceeds 32°C, or the soil temperature exceeds 25°C.
- Avoid high or drying winds.
- Ensure treated area is irrigated both before and after application of ENs.
- Apply ENs as evenly as possible.
- Maintain moist soil conditions for no less than 2 weeks after treatment.

Preventive treatment: Variations occur in the best application timing due to seasonal weather variations or the geographical location of the site. Typically, the first opportunity to apply nematodes targeting African black beetle occurs in December when second-instar grubs appear. Applications should be finished by early February. The first opportunity is referred to as preventive because the larvae are very small at this time and monitoring observations can be misleading. It is therefore better to apply this treatment as a routine part of an annual control strategy.

Curative treatment: Apply nematodes to areas where larvae have begun to damage turf, or at the first signs of bird activity. Damage from scarab beetle larvae can initially be diagnosed as drought stress in turf. Heavily infested areas appear greyish-green, or irregular patches of damaged turf begin to appear.

After application

Apply 12 mm of irrigation immediately after the treatment and continue to maintain high moisture levels for the following 3 days. Maintain adequate moisture levels for the next few weeks. Randomly check grass roots for larvae and reapply nematodes to hot spots.

In hot weather, check the root system of turf that appears healthy but looks stressed when subjected to slight drought stress, or continues to wilt when well irrigated. Good results will be achieved within 7–14 days after treatment.

Steinernema feltiae
Entomopathogenic nematode

Target pests

- Fungus gnats (sciarid flies) *Bradysia* spp.
- Mushroom flies *Lycoriella* spp.
- Currant borer caterpillar (*Synanthedon tipuliformis*)

Plate 85: Fungus gnat adult

Plate 86: Fungus gnat larvae

Adult sciarids are small dark flies 3–5 mm long, with long antennae and a distinctive Y-vein on the wings. They are delicate insects which tend to run across surfaces rather than fly. Females are larger than males. Females mate within a few hours after emerging from pupation and begin laying eggs after a few days. They can lay between 75 and 200 eggs, which hatch in 3–6 days.

The larva (maggot) is translucent white with a shiny black head. The pupa is 3–6 mm long and can be found in the growing medium of pot plants. The life cycle takes 3–4 weeks. The second generation of flies emerges from the compost within 2–3 weeks.

Damage to growing plants from the larvae often goes unnoticed, because they are relatively difficult to find in the medium or within the stem tissue of a plant. The reduction in growth they cause by feeding on the root hairs of a newly planted crop, or the damage to a cutting beginning to grow callus, is hard to quantify. There is often cause-and-effect confusion as to why the plant or cutting has suffered; damage often attributed to a fungal root disease may be the secondary infection and not the primary cause. The chewing larvae provide sites of entry for disease. They can also cause mechanical damage in the form of tunnelling that may collapse the stem, or root damage that reduces water and nutrient uptake.

Damage caused by the adult fly is often overlooked: it acts as an effective vector in carrying pathogens from plant to plant on its feet.

Application

The nematodes are applied with water to the growing medium after planting, as a drench or spray drench, and to any areas in the greenhouse that may be a likely source of infestation. Nematodes perform particularly well in the warm, moist environment of propagation areas.

Using nematodes, best control will be achieved by making the first application at planting or shortly afterwards, or when yellow sticky trap counts are below about 50 per trap per week (guide only). Two or three subsequent applications at weekly intervals may keep fungus gnat numbers low throughout a 10–12-week crop.

Proper application and use of nematodes will vary with the crop and production system. Optimum temperatures for development are between 12°C and 30°C in the medium. At temperatures over 30°C the nematodes become inactive; however, the protected environment of a nursery rarely exceeds this limit within the growing medium.

Recommended application rates

The key to success is the application of routine and preventive treatments. This way, fungus gnat populations are not allowed to build up, and consequent crop damage is prevented.

Preventive treatment: 50 million per 200 m² of growing area. This is equivalent to a rate of 250 000 per m², or 50 million per 5000 × 200-mm pots, since there are 25 × 200-mm pots per m².

Preventive treatments are often based on weekly applications of ENs to protect crops from infestation during the critical early growth stage, or to protect crops grown in conditions that optimise the insect's ability to reproduce.

Inoculation of crops is by far the best approach, in terms of protecting the young plants when they are most susceptible and avoiding the inherent problems associated with the pest. Additionally, lower dosage rates can be used to maintain control, and savings in labour and chemical usage quickly offset the cost of routinely applying nematodes as a biopesticide.

Subsidiary risk areas need to be identified: soil under benches, capillary matting, drains, or the perimeter of soil bins for possible sources of pest pressure. These areas should be included in an overall insect control strategy.

Curative treatment: 50 million per 100 m² of growing area. This is equivalent to a rate of 500 000 per m² or 50 million per 2500 × 200-mm pots, since there are 25 × 200-mm pots per m². Best advice is to initially spray insecticides to lower the adult population; two sprays 3–4 days apart are recommended. If possible the least toxic alternative should be used, to protect any other beneficials that are in the system. Soap sprays targeted at the flying pests rather than the foliage, or natural pyrethroids, have proved effective.

After application

After application, monitor adult fly numbers as well as physically looking for larvae or assessing the damage the maggots are causing. Some confusion can exist, in that the ENs control the larval stage but reinfestation of the growing area may occur from the outside growing environment at certain times of the year. Therefore the effectiveness of the treatment cannot be assessed by numbers of adult flies alone.

A reduction in adult fungus gnat activity should be noted in 2–3 weeks. Susceptible varieties may need an application at the beginning of each growing cycle. Additionally, some varieties may need a preventive treatment during the complete growing schedule.

Cultural practices to aid establishment

Apply ENs when UV levels are at their lowest, because over-exposure to UV light can have a detrimental effect on nematodes at application time. Post-irrigation will help wash any nematodes that have been caught in the leaf canopy back into the growth medium. Aim to wash or apply the solution of nematodes into the top 25–50 mm of medium where the larvae (maggots) will most likely be present.

Apply a knock-down spray application to the adults the day before applying nematodes, and apply follow-up applications to reduce adult numbers twice in the first week if a heavy infestation is present. Use yellow sticky cards to monitor the activity of the adults and to aid in physically trapping and therefore removing adults.

ENs are compatible with hypoaspis mites as part of an integrated pest management program.