Controlling Insect Pests with Entomopathogenic Nematodes©

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INTRODUCTION

Apart from insects, nematodes are the next most common animals on earth with nearly a million different species likely. They are found almost everywhere from the tops of the highest mountains to the depths of the deepest seas. It has been said that if all the rest of the world was removed the ghostly shape of everything would remain as nematodes! Most nematodes are completely harmless but the roundworms that affect our domestic animals and the eel worms that cause billions of dollars damage to crops worldwide are all nematodes. Three of the ten most common diseases of man are caused by nematodes. The largest nematode *Placentanema gigantissima* from the placenta of sperm whales is 8 m long and 2 cm thick and the smallest, *Greeffiella minutum* is only 0.08 mm long and lives free in the sea (Poinar, 1983). However, the nematodes that we use to control insects are about as different from these as human beings are from goldfish.

The first nematode to be used successfully in the control of an insect pest was here in Australia nearly 30 years ago when CSIRO introduced *Beddingia* (then called *Deladenus*) *siricidicola*. This nematode sterilises the sirex wasp, which is the main pest of our 1 million ha of pine forest (Bedding, 1993). Without control it is estimated that the sirex wasp could cause from 1 to 4 billion dollars worth of tree death in every 35-year rotation of these forests. The nematode, which is the main means of controlling it, has prevented this. *Beddingia siricidicola* is used as a classical biological control agent; once introduced into a forest it can more or less be left to look after itself because sirex females transmit it throughout the population. However, the nematodes that can be used to control pests of horticulture are of a very different kind. They seek out and kill various insect pests and are used rather like insecticides (biopesticides), killing insects only near to where they are applied. We call these nematodes entomopathogenic nematodes (ENs). CSIRO was also first in the world to use ENs commercially. This was against black vine weevil in ornamentals from 1981 and against currant borer moth in black currants from 1983. Now various ENs are used around the world against a variety of pests though usually in niche markets (e.g., fungus gnats in plant nurseries, hydroponics, and mushroom houses; weevils on ornamentals, strawberries, cranberries, citrus, and bananas; scarabs of amenity turf, ornamentals and blueberries; cutworms, webworms, billbugs, and mole crickets on amenity turf; termites in houses and trees; peach borer moth in apples in China; carpenter worm in shade trees in China and fig trees in the U.S.A.).

Considerable progress has also been made during the last 20 years or so on the taxonomy, biology, genetics, ecology, host range, production, application technologies, laboratory trials, field trials, and commercialisation of ENs and their symbiotic bacteria resulting in over 2000 publications during this time. This work was briefly reviewed by Akhurst (1996) and more comprehensively by Kaya and Gaugler (1993).

In order to understand how ENs can be used to control pests of horticulture in Australia it is helpful to be aware of what they are, where they are found, how they work, and how best to manipulate them.

ENTOMOPATHOGENIC NEMATODES

Most ENs belong to one of two genera, *Steinernema* of which there are some 18 species described or *Heterorhabditis* with eight species (Akhurst, 1996). The infective juvenile EN is more or less microscopic, anything from 0.5 mm to 1.5 mm long depending on species. It has a closed mouth and anus and cannot feed until it finds an insect. Usually it is found in soil, is activated by insect movement and then follows a gradient of carbon dioxide to find the insect (Gaugler et al., 1980). Now it needs to get into the insects blood cavity in order to kill it. ENs enter through the insect's natural body openings, the mouth, anus or respiratory inlets (spiracles) and then penetrate into the blood cavity from the gut or breeding tubes (Poinar, 1990); *Heterorhabditis* species can also penetrate through chinks in the insect's armour (the interskeletal membranes) by scratching away at these with a special tooth (Bedding and Molyneux, 1983).

Once in the insects blood the EN infective juvenile releases a highly specialised symbiotic bacterium found only in ENs (*Xenorhabdus* spp. in *Steinernema, Photorhabdus* spp. in *Heterorhabditis*). These symbiotic bacteria multiply and rapidly kill the insect within a day or so. The bacteria then convert the insect into suitable food for the nematodes and produce a range of antibiotics (Akhurst, 1982) and anti-feedants that preserve the dead insect while the nematodes feed and breed up within it. After about 10 days a medium-sized insect cadaver may produce up to 100,000 or more infective juvenile ENs that are released into the soil and seek out new insect pest hosts. Unfortunately there are many fungi and other organisms that can attack the infective juveniles before they enter an insect pest (Kopenhoffer et al., 1996, 1997).

OCCURRENCE AND DISCOVERY

ENs have been found in soils throughout the world using the wax-moth baiting technique of Bedding and Akhurst (1975). This technique involves simply taking soil samples of about 0.5 kg comprising 5 to 10 subsamples over an area of a few square metres, filling plastic food containers with the soil, adding five wax moth larvae to each, replacing the container lid and then inverting the container. The wax moth larvae are so susceptible to ENs that they are killed even if there are only half a dozen or so ENs in the soil. ENs can then be bred out of the dead wax moth larvae. Using this technique we (Akhurst and Bedding, 1986) and others (Hominick and Briscoe, 1990; Yoshida et al., 1998) have found as many as one in three soil samples taken in this way from various countries and habitats contain ENs. Doubtless most of you will have at least one species of EN on your property. Unfortunately they are usually poorly infective strains, poorly distributed, and in such low numbers that they have little affect on pest insect populations.

To use ENs in insect control we select the best species and the best strain of that species, produce it in vast numbers and apply evenly so that larger numbers of ENs can reach each insect pest and ensure there is a water film between pest and ENs.

SAFETY

Various tests against mice, rabbits, and monkeys (Gaugler, 1979; Wang et al., 1983, 1984; Wang & Liu, 1983; Boemare et al., 1996) have shown that the ENs tested are harmless when fed, injected, or inhaled. They are also harmless to earthworms (Capinera et al., 1982) and other non-insect organisms including plants and they are of course nonpolluting. They have now been used on a large scale in various countries for over 10 years and large numbers of production workers have been exposed to thousands of billions of them with out any adverse effects being recorded. The EPA in the U.S.A. and many other countries including Australia have exempted ENs from registration. However as with a variety of materials from pollen and flour to various insects and plants there is always the possibility of some individuals developing allergies to ENs so that it is wise to prevent the possible inhalation of EN sprays and skin contamination with them.

PRODUCTION

It is far too expensive to rear ENs inside insects at least in industrialised countries. CSIRO pioneered the use of the ENs own symbiotic bacteria to convert various crude media into ideal food for culturing ENs aseptically on the three-dimensional carriers (Bedding, 1976, 1981, 1984). Essentially we use crumbed waste upholstery foam to provide a large surface area on which a thin layer of media is distributed. This is sterilised by autoclaving and then aseptically inoculated with the symbiotic bacteria of the nematode to be cultured. Aseptic nematode inoculum is added after a few days of incubation and 2 to 3 weeks later nematodes can be harvested and processed using specialised apparatus. We have patented (Bedding et al., 1992) culture chambers that are self-aerating while maintaining uncontaminated nematode/bacterial growth throughout the culture period and used these to produce many hundreds of billions of ENs for trials and commercial use.

FORMULATION AND STORAGE

It usually takes about 2000 million ENs to treat 1 ha. Because ENs are living organisms requiring a continual oxygenation, it is impractical to supply them to growers in a water suspension that would have to be kept continually aerated and would even then only last for a few days. Temporary refrigerated storage can be achieved by adding cream of nematodes to crumbed foam in plastic bags (Bedding, 1984) but the grower has to extract these from the foam. However we have patented a number of better ways of formulating ENs (Bedding, 1986; Bedding and Butler, 1993; Bedding et al., 1998). The latest formulation is comprised of about 50% micro-cellulose and 50% nematodes that can be readily mixed in spray tanks, sprayed without blocking nozzles, and can survive several months at room temperature. This long shelf life has been achieved by manipulating the nematodes' physiology.

Water is removed from between the nematodes over a filter under vacuum and then more water (about 50%) is removed from within the nematodes. This causes the nematodes to produce up to 10% sugars (glycerol and trehalose) within them (candied nematodes) and enter into hibernation. When in hibernation they may use only one hundredth of the oxygen of nonhibernating nematodes and in theory at least can last 100 times as long. One problem was that by this time fungal contamination develops and it has been very difficult to find a suitable preservative that does not harm the nematodes but keeps fungus at bay for several months.

Another problem was that the ENs must be kept at a precise water content for maximum shelf life but it is difficult to provide them with oxygen while preventing water loss.

USING ENTOMOPATHOGENIC NEMATODES

Entomopathogenic nematodes are now being used commercially to control a number of insect pests and recently at 13% of sales of bio-insecticides in industrialised countries they were already second only to *Bacillus thuriengiensis* at 80% (Lisansky and Coombs, 1994).

Our current EN formulation can be simply added to water and then agitated before and during spraying but unfortunately cannot be applied quite as simply as insecticides. Being living organisms they are subject to damage from UV light and from desiccation. They will also sediment in spray tanks if not agitated properly. And, they need a water film to move through to reach their targets. Thus when applying to soil to treat various pests, the soil must be moist though not too wet, and even the extreme surface needs to be moist. When applying ENs, the area to be treated needs to be carefully watered just before. Because ENs are susceptible to UV light they should never be applied during sunlight and preferably at dusk. Even though the ENs can move to seek out insect pests they cannot go very far (just a few cm) and the nearer you can get them to the insect the more likely they are to be able to infect that insect. Essentially every sq cm of soil surface should receive its quota of ENs. However, failing this, particularly for potted plants, ENs can be sprinkled immediately over the root zone. After spraying ENs onto the soil surface it is advisable to irrigate that surface again. This helps to wash the nematodes down through the vegetation and even partly into the soil. Another problem affecting nematodes is temperature extremes. ENs cannot infect at temperatures below about 12°C and many kinds of them are stressed at temperatures over 30°C. In some cases they can however survive, without being able to infect, at temperatures below freezing.

On a small-scale ENs can be applied using hand spray drenching on potted plants or venturi sprayers such as those used for “weed and feed” for amenity turf. On a larger scale properly adapted irrigation systems are used on seedlings and potted plants (for instance see methods developed by Trevor Bullen on www.bullensnursery) and boom sprayers on amenity turf.

EXAMPLES OF USE

Currant Borer Moth. One of the first insects to be controlled commercially using ENs was the black currant borer moth, *Synanthedon tipuliformis,* the caterpillars of which bore through the stems of blackcurrants often halving the yield and also halving the vitamin C content of the remaining berries (Bedding and Miller, 1981; Miller and Bedding, 1982). We aimed to disinfest cuttings used to establish new plantations as well as to treat already established plantations. Over a million cuttings were stacked in walls and sprayed all over with a concentrated suspension of *Steinernema feltiae* infective juveniles. The cuttings were then covered with plastic sheeting to maintain humidity for several days and later sampling showed that nearly 100% of the larvae had been killed and it was calculated that therefore it would be many years before currant borer moth populations built up in the new plantations to significantly damaging levels. In addition, several plantations that already had serious infestations of currant borer moth were treated by blast spraying the currant bushes with a suspension of *S. feltiae* infective juveniles and this reduced borer moth populations by over 70% (Miller and Bedding, 1982a,b). An important factor here was to spray during times of very low wind otherwise the nematodes had difficulty detecting carbon dioxide emerging from holes made in the stem by the adult moth during egg laying and the ENs also dried up too quickly.

Black Vine Weevils. The black vine weevil, *Otiorhynchus sulcatus*, is a major pest of the potted plant industry worldwide, particularly of cyclamen, begonias, and impatiens but several hundred other plant species are also susceptible. Azaleas, rhododendrons, strawberries, cranberries, and other berry fruit are affected both under cover and outdoors and this insect is purported to be the worst garden pest in the UK at least.

Entomopathogenic nematode control of black vine weevil was first developed and commercialised in Australia and, with control of black currant borer here, represented the world's first commercialisation of ENs.

Entomopathogenic nematodes, particularly *H. bacteriophora*, are now used widely for control of black vine weevils in Europe, the U.S.A., and Australia when soil temperatures are at least 12°C.

Heterorhabditis zealandica X1 strain is superior to *H. bacteriophora* but won't work effectively at temperatures below 15°C.

Sciarids—Fungus Gnats. Sciarids are serious pests of seedlings, many hydroponically grown plants, and mushrooms worldwide. The larvae of *Sciara* species feed on roots and adults transmit disease while larval *Lycoriella* species are the most important insect pests of the mushroom industry.

Entomopathogenic nematode control using *Steinernema feltiae*, developed in the UK, is now widely used in Europe and U.S.A. and has recently begun in Australia. The nematodes are usually applied using overhead sprinklers.

Banana Weevils. As an example of how ENs can be manipulated to control even insects, which are the least susceptible to them, consider the banana weevil, which is one of the most important pests of bananas. Even though adults of this insect can each be covered with thousands of ENs without becoming infected, we (Treverrow and Bedding, 1993) were able to develop a system that resulted in nearly 100% mortality of insects coming to bait: briefly, the weevils are attracted to damaged banana tissue and will remain in crevices. Entomopathogenic nematodes cannot penetrate the weevils through the long proboscis or through the anus, which is tightly closed, and they cannot get into the breathing holes (spiracles) because these are tightly covered with the wing cases. Our final solution (after lots of attempts) was at the time of harvesting when the tree is cut down, to have the grower: (1) Using a desuckering tool, cut out a cone of banana tissue from the residual corm; (2) To place in the resulting hole, polyacrylamide gel crystals together with ENs and 1% mineral oil; (3) To replace the cone of banana tissue. The polyacrylamide gel absorbs sap from the hole, which would have stopped the ENs working and provides a large surface area from which the ENs can contact the weevils. Replacing the cone encourages attracted weevils to remain. The 1% oil smears at the edge of the wing covers reducing air intake and the weevil has to lift its wing covers whereupon ENs can easily enter the spiracles.

Caterpillars. Entomopathogenic nematodes have even been used against leaf eating insects; thus Begley (1986) successfully developed use of *S. carpocapsae* against *Spodoptera* in the largest chrysanthemum establishment in the U.S.A. (Yoder

Brothers). The plants were grown under shade cloth that maintained humidity and the ENs were simply sprayed in a water suspension at dusk when humidities were near to 100%. *Spodoptera* spp. and *Heliothis* spp. are feasible targets but ENs have not yet been tested against them in Australia. Try them!

Carpenter Worm in Shade Trees. A carpenter worm, *Holcocerus insularis* is the major pest of trees such as ash (*Fraxinus pennsylvanica*), the Chinese scholar tree (*Sophora japonica*), and willows (*Salix* spp.) that are planted to provide shade for pedestrians and cyclists in the streets of cities in northern China. Hundreds and sometimes thousands of caterpillars eat out the inside of these trees. In 12 cities affected by *H. insularis*, 30% to 80% of shade trees are infested and 5% killed annually. Now instead of fumigating the trees, which is quite complicated and dangerous, council workers inject uppermost holes with a suspension of *S. carpocapsae*. The ENs kill many of the caterpillars after a few days; then nematodes breed up within the cadavers and the next generation kills the remainder (Yang et al., 1993). Well over 10 million trees have now been treated and the pest has been almost eliminated from several large cities.

Scarabs. Larvae of various species of scarabs (white grubs, curl grubs, cockchafers) feed on the roots of a wide range of plants. After many years of searching CSIRO have finally found and patented use of strains of an EN species, *H. zealandica* X1, that are particularly effective against scarabs. Most of our trials and over 800 commercial treatments have been against scarabs in amenity turf, but we have found this EN to be effective to most of the scarab species that we have tested and would expect it to be equally effective against scarabs attacking other plants. However, it is only poorly infective against Christmas beetles (*Anoplognathus* species) and not infective to pruinose scarab (*Sericesthis geminata*).

Western Flower Thrips. The possibility of controlling this insect with ENs is a bit of a long shot. However, Tomalak (1994) working in Poland found that he could get high levels of kill of Western flower thrips pupae, in the laboratory, with one strain of *S. feltiae* selected from among 100 of that species. One attribute of this strain was the thinness of the infective juveniles. We believe it may be possible to achieve this thinness with any strain of *S. feltiae* by manipulating feed back mechanisms during artificial culture.

Stem Borers. As in the case of black currant borer, other moth borers such as fruit tree borer, *Maroga melanostigma*, occasionally found in raspberries could be controllable with ENs. However, some beetle borers such as the elephant weevil, found in blueberries and grapes, pack their frass so tightly behind them when they bore into stems that it is impossible for the nematodes to pursue them.

COMMERCIAL TREATMENTS 2001/2002

CSIRO has licensed its EN production, formulation, application technologies, and EN strains to Ecogrow Australia Pty Ltd. During the last year Ecogrow has utilised some 15,000 kg of sterile media to produce over 3 trillion nematodes that have been used in over 1000 successful commercial treatments (up to 10 ha) for turf and horticulture. So far the main insect targets have been African black beetle, Argentine scarab, red headed cockchafer, Argentine stem weevil, billbug, fungus gnats, and black vine weevil.

CONCLUSION

The use of ENs is really still in its infancy and there is considerable potential yet to be fulfilled (Bedding, 1999). New species and strains of ENs are constantly being found and can now be stored in liquid nitrogen indefinitely to preserve genetic diversity (Popiel and Vasquez, 1991; Curran et al., 1992). Most of our domestic animals and plants have been modified by artificial selection and this is undoubtedly possible with ENs (particularly because of their short life cycle) and has indeed been already attempted with some success (Tomalak, 1994; Grewal et al., 1996; Glazer et al., 1997). As better and better strains become available, more kinds of insect pests can be targeted and fewer ENs will be required for treatments that will therefore become less expensive. However ENs have little prospect of controlling such pests as two spotted mite, aphids (except possibly subterranean aphids), and sucking bugs such as Nezara. This is because generally ENs work best on pests that spend at least some of their life in the soil or other cryptic habitats. There is also much research being conducted on methods for applying ENs that should help to further reduce treatment costs. However, in the end in using ENs to control a variety of pests it may be partly up to the ingenuity of the grower to find out the best possible means of applying them for his/her particular situation, appliances, and crop.

The advantages of using these biopesticides are in operator safety; they are harmless to humans, domestic animals, earthworms, birds and plants; they are quick and easy to handle and apply; there are no resistance problems; there is no withholding period; there is minimal harm to natural enemies and beneficial microbes; no environmental pollution; or disposal problems and there is a good public image. Give them a go!

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The Evolution of Container Design©

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INTRODUCTION

While plants have been grown in containers for many thousands of years, formal design of plant containers is more recent. The most rapid developments in container design occurred in association with the rapid development of container production that occurred after WW II. Changes in nursery practice, increased research into plant growth, and plastics manufacturing technology all contributed to the changes in design that have occurred in the past 50 years. In this paper, most emphasis will be placed on containers for woody plant production because of the well-documented deleterious effects that poor practice can have on root system quality in trees and shrubs.

WHAT IS CONTAINER DESIGN?

In order to trace an evolution of container design in nursery plant production, we have to initially define what we mean by design. Design is generally held to involve a deliberation of thought in order to bring about a particular end or function. If one takes the view that container design occurs when a set of objectives are set out, and a process is undertaken to ensure that these objectives are met before the container is made, then container design, especially for woody plants, can probably be dated to relatively recently.

In any modern container design process, it seems to me that several criteria should be imposed. These are: Does the container grow the plant in such a way that there is no impairment of growth, either in the nursery or subsequently; can the container be manufactured; is the cost of the container low enough that it can be used economically; does the design allow for easy uptake by the nursery industry? Experience and observation would suggest that unless all of these criteria are met, uptake is unlikely irrespective of how good the container is. Unfortunately, the first of these criteria has often not been taken into consideration when the design process has been undertaken.