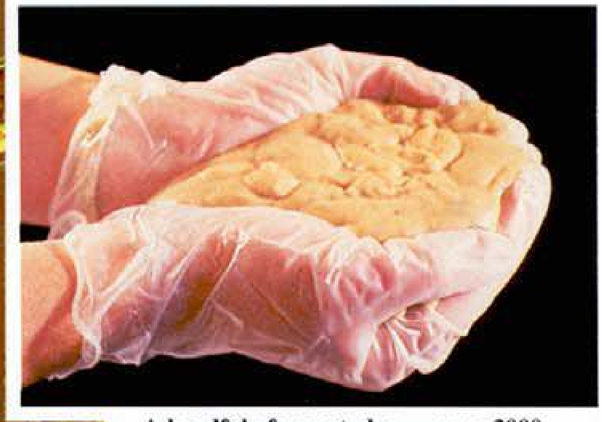
An infective juvenile nematode (*Steinernema feltiae*), about half a millimetre long. This species is the one most commonly available from commercial sources. It is widely used in the United States for treating termites in houses, and shows a good potential for controlling banana weevil in Australia.



A handful of nematodes — some 2000 million or so. They are juveniles of *Steinernema bibionis*, which infect blackcurrant borers.

Pesticides — the nematode alternative

Worms, billions upon billions of tiny worms called nematodes, may soon be used as a biological control agent against many types of insects.

Dr Robin Bedding of the Hobart laboratory of the CSIRO Division of Entomology has been working with colleagues on ways of mass rearing them so that nematode sprays can be economically produced. He is now able to produce nematodes at a price that he believes will make these sprays a practical alternative to chemical sprays.

In April this year, Dr Bedding's team produced 60 000 000 000 tiny worms for growers to spray on 30 ha of blackcurrants — the first commercial-scale operation of this sort. The nematodes, *Steinernema bibionis*, each only about 0·6 mm long, are parasites on the larvae of the currant borer moth, *Synanthedon tipuliformis*, and soon search out their hosts and kill them.

In earlier trials conducted by CSIRO in conjunction with the Tasmanian Department of Agriculture, 90–95% of the borers succumbed: a better rate than is usually obtained with chemical spraying, where an 80% reduction in larval numbers is considered a good figure. Its cost is comparable to the expense of applying chemical pesticides, but it leaves no poisonous residues on the fruit and doesn't interfere with the life cycles of most beneficial insects (in particular, bees and the predators of the two-spotted mite).

Extensive testing has shown that these nematodes will not harm man or other animals (they are closely related to familiar intestinal parasites).

Currant borer moth is difficult to control with chemicals because the larvae burrow inside the blackcurrant canes. Commonly, the larvae are found some 20 cm from a tiny entrance hole, and behind a barrier of frass. To be effective, pesticides must be sprayed on the eggs while they sit exposed on the outside of the canes, but timing of the application is critical. No entirely satisfactory chemical control methods are available.

Earlier trials by Mr Bob Hardy of the Tasmanian Department of Agriculture showed that the borer moth could cause losses of up to 30% of the potential blackcurrant yield. Borer tunnels, up to 40 cm long, seriously weaken the stems, and these sometimes break. More significantly, afflicted canes carry fewer flowers, and fruit. Also, the remaining fruit produce less vitamin C, losing up to half the normal quantity — and much of Tasmania's blackcurrant crop goes to make a high-vitamin-C cordial.

Mr Hardy and colleagues have found that, despite chemical spraying programs, the average plantation is infested with one larva for each 5 m of blackcurrant cane.

Since currant borer is an insect that doesn't travel far and produces relatively few offspring, Mr Laurie Miller of the Tasmanian Department of Agriculture calculated that if a population infesting a particular plantation could be hit with a 99·5% mortality rate, then the plantation's level of infestation should remain below one-tenth of the Tasmanian average for at least 6 years.

Dead reckoning

To achieve a high level of mortality with the nematode technique, each blackcurrant bush is sprayed with about a litre of water containing perhaps 1 or 2 million nematodes. The worms' natural habitat is the soil, which prevents them from drying out. Finding themselves on the surface of the cane, they have but a few hours to find their host before they dry out.

Unhappily for the borer, the worm actively seeks out the entry holes, like a ferret after a rabbit, and the researchers found in one test that more than 1000 nematodes can enter a single tunnel a short time after spraying. Following up some days later, they examined some tens of bushes and found that about 90% of larvae had been killed by nematodes.

The nematodes enter their target through the mouth, anus, or breathing holes. One species studied, *Heterorhabitis bacteriophora*, possesses a special tooth that it uses to burrow through the weak chinks of an insect's armour (the worm is only 8–15 μm in diameter). It can then penetrate the body cavity and release a highly specialized bacterium, which proliferates and kills the insect by septicaemia.

Because of the importance of these bacteria, Dr Ray Akhurst of the CSIRO Division of Entomology has centred his research on them.

Each of the nematode species has its own special symbiotic strain of bacterium. Newly hatched nematodes may carry a wide range of microbes, but only the one that

Inside a blackcurrant cane, a currant borer has been pursued and killed by the nematode *Steinernema bibionis*.

Tasmanian blackcurrant bushes getting the treatment. Each bush is dosed with a water spray containing millions of nematodes.

gives the infective nematode an advantage gets to survive in its intestine. For the nematode *Steinernema* sp., the symbiotic bacterium is a *Xenorhabdus* species.

It penetrates the body cavity and releases a highly specialized bacterium.

Xenorhabdus benefits from the arrangement: it is transported to a host that it can multiply in. For its part, the nematode is provided with partly digested food that fulfils essential nutritional requirements. (The nematode cannot live without the bacterium: it's as if it had a weak digestive system that needs a special yoghurt culture to help it along.)

In addition, *Xenorhabdus* produces broad-spectrum antibiotics that preserve the insect cadaver from putrefaction. In this way, the body of an insect can be preserved for weeks as food for the nematode, just as yoghurt cultures can keep milk palatable for us for an extended time.

After entering as a juvenile, the nematode matures and reproduces inside the dead insect, and after the eggs hatch the young (complete with symbiotic bacterium) leave to find another host. A medium-sized insect may produce up to 100 000 new infective juveniles. Provided they have sufficient water, these new nematodes can survive for many months, perhaps years, without feeding. Normally, they wait in the soil for an insect that normally lives there or is about to pupate there. (However, pupae are resistant to nematode attack, and so control of pupating pests isn't as easy as it seems: it needs precise timing, and nematodes themselves are susceptible to attack by fungi.)

Mass rearing

Of the hundreds of insect species that scientists have used in tests with *Steinernema*, nearly all have, in varying degrees, proved susceptible to it. If an insect is penetrated by a nematode, it will be killed, but particular insects differ in their resistance to penetration. However, there seems no reason to think that these nematodes cannot be used to control a very wide range of insect pests. The general approach is to overcome the naturally poor dispersal ability of the nematode by mass rearing and spreading them artificially. (In their normal environment, the soil, they may not move more than a metre or so in a year.)

Two problems loom large when nematodes are sprayed onto crops. The first is to maintain moist conditions, or very high humidity, for long enough to allow the creatures to reach their target before they dry out — they have very low resistance to desiccation. The second is the cost of breeding enough nematodes; and obviously, the longer the nematodes survive, the fewer will need to be bred and applied.

In Dr Bedding's field test, the nematodes were applied in water droplets, but of the millions sprayed on each bush, only some thousands made it to the moist environment within a borer hole. The spraying was done on calm days during or after light rain. Poor results were obtained when it was windy and the relative humidity fell below 70%, when temperatures fell below 11°C, and when the nematodes were suspended in small volumes of water.

Dr Bedding thinks that nematode survival could be improved by procedures such as spraying at night, misting crops from irrigation sprinklers, covering crops with plastic sheeting, and adding water thickeners and evaporation retardants to the spray. He

A female blackcurrant borer moth.

did some work with encapsulating nematodes in oil and wax droplets, which kept them alive for long periods, but the tiny worms found it difficult to escape.

Although a number of scientists have claimed success in spraying *Steinernema* onto crops, the cost factor has so far been the major obstacle to commercial use of nematodes. A lot of the expense can be attributed to the use of host insects to rear the nematodes, or the use of expensive substrates.

One researcher in 1972 worked out a system of rearing nematodes on dog biscuits with one technician being able to produce 100 million infective juveniles per week. Dr Bedding reckons that, using the technique he has developed, one person could produce 30 000 million *S. bibionis* per week.

The key to economy is to grow in bulk the symbiotic bacteria associated with the nematodes. They are capable of converting a wide variety of substances into 'babyfood' digestible by the nematodes. Previous workers who have used artificial media relied on preservatives to inhibit growth of other bacteria, or on very heavy inoculations to swamp any contaminating organisms. Consequently, yields were low.

Dr Bedding's technique relies on using a sterile medium that is inoculated with the desired *Xenorhabdus* bacterium. He has tried a number of media — including soybean, heart, liver, kidney, meat meal, and blood meal — but the best and cheapest has been homogenized chicken offal. Several kilograms of it are coated onto crumbs of waste upholstery foam within sealable plastic bags. The idea is to provide as large a surface area in as small a volume as possible, and still provide plenty of aeration for the worms' metabolism. After sterilization, the bags are inoculated with symbiotic bacteria and filtered air supplied to them.

Five days later, nematodes are added, and after some weeks the crop can be harvested. Each bag can produce 1000–2000 million nematodes.

Provided the harvested nematodes are kept aerated, they can be kept alive in non-sterile plastic bags at 1–2°C for a number of months with less than 10% mortality.

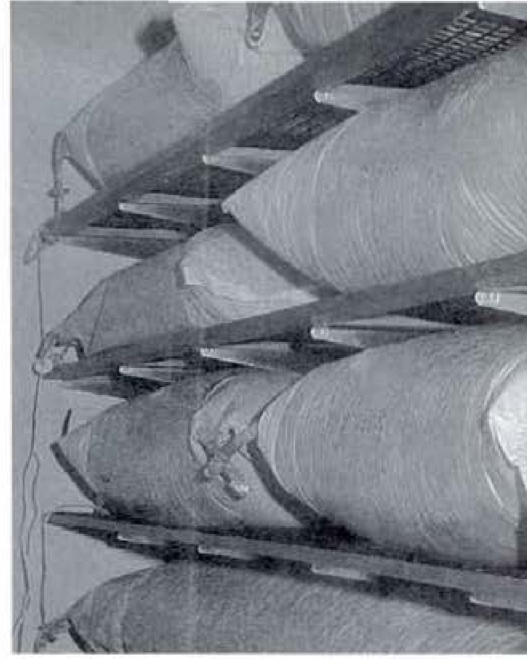
Dr Bedding's method has been patented, and an Australian company, Biotechnology Australia Pty Ltd, has a licence to produce nematodes for major international markets. The company now has some 15 highly qualified people perfecting the art of raising nematodes, which it is supplying for local and overseas use.

Disinfest cuttings

As well as using *Steinernema bibionis* for spraying on blackcurrant crops, it is also worth while using it to disinfest cuttings that will be planted out in new plantations. Dr Bedding and Mr Miller worked together in 1980 to establish the feasibility of this technique.

Whenever growers wish to extend their plantings, they use fresh cuttings from existing bushes. As we have noted, if a plantation can be established from borer-free material, and it is some distance from a source of infection, then it should remain borer-free for many years. Insecticidal dips are ineffective in controlling the pest.

The researchers found that *S. bibionis* could cause 99% mortality in commercial consignments of canes. The superior performance (compared with field application) results from the ease of maintaining moist conditions. The bundles of canes are stacked together with damp paper, sprayed with nematodes, and enclosed in large plastic bags.



Incubating nematodes by the bagful. Each culture bag can breed up 1000 million or more nematodes.

The method has now been used on more than half a million cuttings planted throughout Tasmania and in some parts of Victoria.

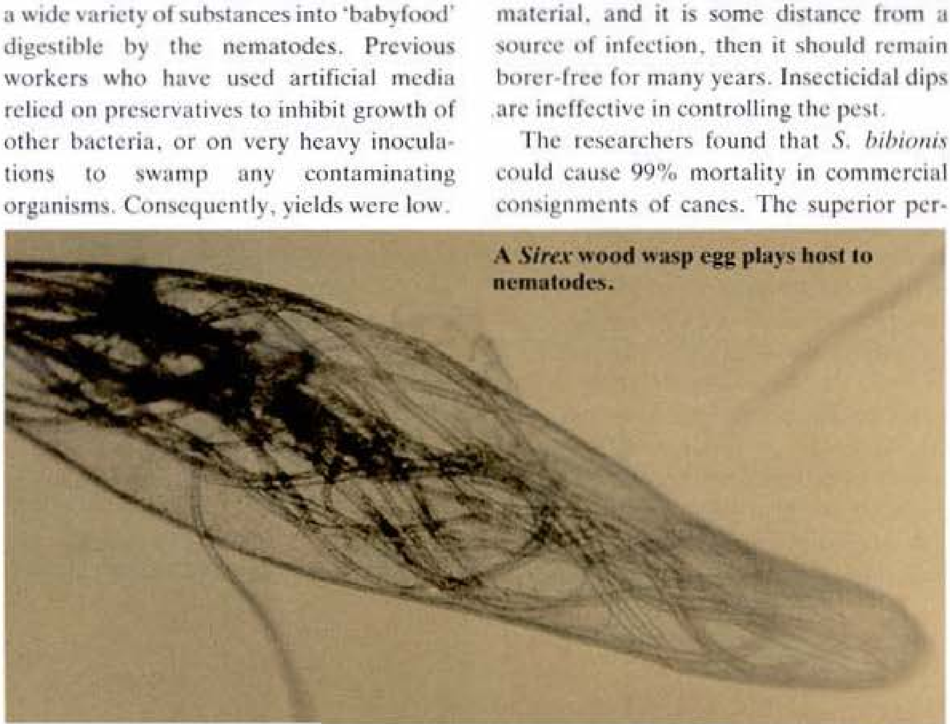
Sirex

Another major success for Dr Bedding's team was the case of the *Sirex* wood wasp. Indeed this project was the first in the world to successfully employ nematodes as biological control agents. The wasp once threatened Australia's pine plantations, but the CSIRO team, from a study of the biology of an extraordinary nematode, *Deladenus siricidicola*, worked out how *Sirex* could be controlled.

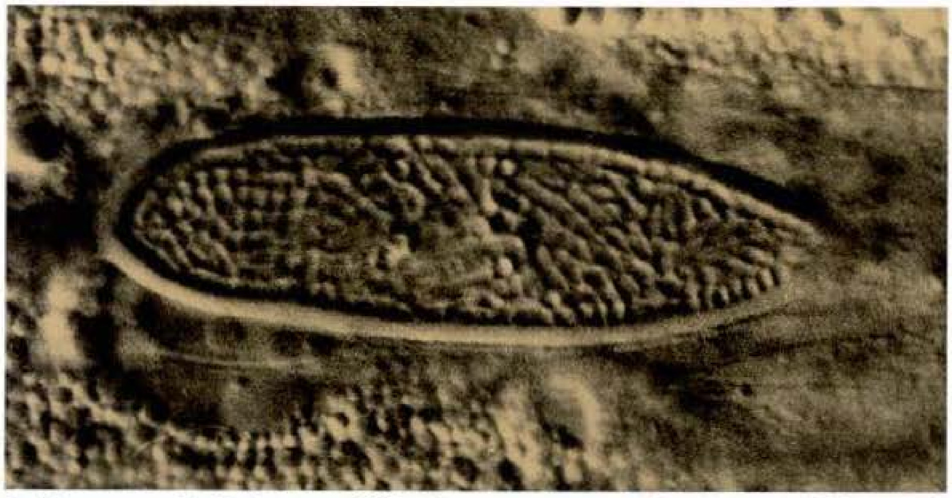
The team developed methods of mass rearing and distributing the nematode, which infects the larvae of wood-burrowing *Sirex* wasps. The scientists made up a medium containing thousands of nematodes per millilitre, which could then be injected into trees in *Sirex*-infested regions.

As the infected *Sirex* larvae grow into adult wasps, the infection is carried to the female's eggs. When she injects her eggs into the wood of pines, she deposits a special fungus with them that is intended to provide food for the emerging grubs. However, instead of *Sirex* grubs emerging from the eggs, nematodes do so. They take the opportunity to feed upon the fungus, and breed up in vast numbers.

They soon spread through the tree and infect most of the progeny of healthy *Sirex* that often attack the same tree. Parasitized



A *Sirex* wood wasp egg plays host to nematodes.



Inside a nematode *(Steinernema bibionis)*, showing the vesicle containing its symbiotic bacteria.

adult *Sirex* emerge and spread nematodes to other trees throughout the forest.

In co-operation with the Forestry Services of Tasmania, Victoria, and New South Wales, *D. siricidicola* has been liberated throughout 200 000 hectares of pine forest. It is now considered to be the main controlling agent of *Sirex*.

Pot plants

A more recent success came with the finding of a nematode that attacks a serious pest of many horticultural crops, the black vine weevil. Otherwise known as the European strawberry weevil, *Otiorhynchus sulcatus*, it attacks more than 140 plant species, including strawberries, grapes, blackcurrants, cyclamens, begonias, and rhododendrons.

The weevil larvae feed on the roots and reduce the vigour of the plant, even killing it. Adult weevils feed on foliage, and cause unsightly damage to ornamentals.

Again in co-operation with the Tasmanian Department of Agriculture, the CSIRO team achieved almost 100% mortality of the weevil larvae infesting raspberries, yews, and grapes, and more than 90% mortality in the cases of potted cyclamens and strawberries. Affected plants were drenched with a suspension of *Heterorhabditis heliothidis*.

Disinfesting blackcurrant cuttings by spraying nematodes on them.

Recently some 100 000 potted plants in commercial nurseries, and many in the Hobart botanical gardens, were quickly and effectively sprayed. Field tests with strawberries, in which the nematodes are injected into the root zone, also look very promising.

Ecological factors

A noteworthy feature of this work has been the different performances of various strains of nematode. One strain, isolated from Tasmanian soil, was particularly susceptible to temperatures below 12°C. This emphasizes the importance of selecting not only the most infective nematode, but also the one best adapted to survival in the field.

Dr Bedding and Dr Akhurst have developed a simple method for detecting and isolating nematodes from soil. In outline, it entails baiting a jar of soil with larvae of the greater wax moth, *Galleria mellonella*, and examining the nematodes that infect them. Since *Galleria* larvae are not normally exposed to nematodes (in Nature, they don't come in contact with soil), they are far more susceptible than soil-inhabiting insects that have evolved degrees of protection.

Galleria seem to exude some scent that attracts virtually every nematode in the sample. In this way even very sparse nematode populations — perhaps but a single nematode per kilogram of soil — can be detected.

The scientists have used this method to find several undescribed species of *Steinernema* and one entirely new genus.

Studies with various species and strains have shown that no one nematode excels in

A black vine weevil grub killed by nematodes.

infecting all insect hosts. One type is best suited to a certain insect; another is most effective for some different host. In particular, it seems that a nematode is unlikely to be the most infectious agent against its natural host — continued association has led to selection for decreased infectivity. Just as a well-evolved parasite does as little harm to its host as possible, the nematodes (which are parasitoids — they kill their host) have probably evolved to do as little harm to the host population as possible.

Therefore, in looking for a nematode to control an insect population, it may often be best to use a relative stranger rather than the nematode naturally associated with the insect. Members of the CSIRO team are looking at factors responsible for how infectious a nematode is. Evaluating a nematode's degree of attraction, speed of movement, penetration mechanism, and the defence mechanism of the host may allow the scientists to predict which nematode will be most infectious for a particular insect.

Hither and yon

Casting further afield, Dr Bedding and his colleagues are doing work in Victoria and South Australia, as well as Tasmania, on the feasibility of using a species of nematode against red-headed cockchafer, which has done considerable damage to pastures in those States.

The scientists have also begun experiments on controlling banana weevil, which appears to be a promising candidate for nematode control. Additionally, they are looking into the possibility of afflicting sheep blowflies and giant termites.

Australia and China have been collaborating in nematode research for some years, and at the moment ways to strengthen this collaboration are being explored. The Chinese are extremely interested in this method of biological control, and have set up large research establishments at Guangzhou and Beijing to look at its feasibility for a wide range of pests, including cotton bollworm, sugar-cane borer, rice stem borer, and cabbage white butterfly. Chemical control of these pests is expensive, but the low labour cost in China should make the rearing and broadcasting of nematodes (which is labour-intensive) economically favourable.

For Australia's part, such collaboration will be beneficial because it will allow very large field trials to be conducted and evaluated.

Nematodes look like taking on an important role in normal agricultural practice.

Andrew Bell

More about the topic

Field testing of the insect parasitic nematode, *Neoaplectana bibionis* (Nematoda: Steinernematidae) against currant borer moth, *Synanthedon tipuliformis* (Lep.: Sesiidae) in blackcurrants. L.A. Miller and R.A. Bedding. *Entomophaga*, 1982, **27**, 109–14.

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A simple technique for the detection of insect parasitic rhabditid nematodes in the soil. R.A. Bedding and R.J. Akhurst. *Nematologica*, 1975, **21**, 109–10.

Heterorhabditis spp., *Neoaplectana* spp., and *Steinernema kraussei*: interspecific and intraspecific differences in infectivity for insects. R.A. Bedding, A.S. Molyneux, and R.J. Akhurst. *Experimental Parasitology*, 1983, **55**, 249–57.

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