21 Application of *Beddingia siricidicola* for Sirex Woodwasp Control

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21.1. Introduction

The use of the nematode *Beddingia (Deladenus*[1]*) siricidicola* is now recognized as the most important means of controlling *Sirex noctilio*, a serious pest threatening nearly 8 million ha of pine plantations in the southern hemisphere (Iede *et al.*, 2000; Carnegie *et al.*, 2003).

S. noctilio (Hymenoptera: Siricidae), originally from Europe, is the only one of some

[1] Those species of *Deladenus* having both free-living and parasitic life cycles were assigned to a new genus, *Beddingia*, by Blinova and Korenchenko (1986) and this nomenclature was adopted by Remillet and Laumond (1991).

 Nematodes as Biocontrol Agents (eds P.S. Grewal, R.-U. Ehlers and D.I. Shapiro-Ilan)

40 species of woodwasp found throughout the world that can kill relatively healthy pine trees. The tree species most susceptible to sirex, *Pinus radiata*, *P. taeda*, *P. elliottii* and *P. patula*, all of which originated from North America, were long ago adopted for major plantations in Australia, now with 1 million ha; New Zealand, 1.8 million ha; Brazil, 2.2 million ha; Chile, 1.5 million ha; Argentina, 0.3 million ha; Uruguay, 0.1 million ha; and South Africa, 0.7 million ha (Iede *et al.*, 2000; Wood *et al.*, 2001; M. Wingfield, 2003, personal communication).

Later, *S. noctilio* was accidentally introduced into each of these regions (New Zealand during the early 1900s, Tasmania during the early 1950s, the mainland of Australia at the beginning of 1960s, South America during the 1980s and South Africa during the 1990s) so that there is now an unfortunate combination of the most virulent woodwasp, highly susceptible tree species, sometimes high density of plantings with inadequate forest management, originally an absence of natural enemies and, at least in Australia, a climate disposed to make trees periodically even more susceptible to attack.

Initially, during the early 1960s, it was hoped to eradicate sirex on the mainland of Australia by having mandatory reporting and by seeking out and destroying all sirex-infested trees; none the less, sirex spread at a rate of about 20–30 km/year, reaching borders of the state of Victoria after about 20 years. Sirex has now spread to the major part of Australia's 1 million ha of pine plantations, reaching most plantations in Tasmania, Victoria, New South Wales and South Australia and has just (Carnegie *et al.*, 2003) been found near the border of Queensland, but not yet in Western Australia. In South America, sirex is now well established in Uruguay, Brazil and Argentina (Iede *et al.*, 2000) and was reported from Chile in 2001, while in South Africa it has recently migrated from the Cape to near Durban (M. Wingfield, 2003, personal communication).

B. siricidicola was first introduced into the Australian state of Victoria during the early 1970s and from then on there were relatively few serious outbreaks of sirex. This led to complacency so that even though sirex arrived in the 113,000-ha 'Green Triangle' forests of southwest Victoria/southeast South Australia during 1979, no serious attempt was made to introduce nematodes for the next 8 years (Fig. 21.1). By then it was almost too late; in 1987 1.8 million trees were killed by sirex and during the next 2 years a further 3 million were killed. This area was thus rather like a huge control plot showing that in the absence of natural enemies, sirex could kill up to 80% of trees in some areas. Fortunately, as a result of an AUS$1.3 million operation to introduce nematodes during 1987 (Haugen and Underdown, 1990a), levels of nematode parasitism of up to 100% were reached within 2 years and the sirex population crashed, but not before millions of dollars worth of timber was lost and the quality of many of the remaining trees impaired (M.G. Underdown, 1992, personal communication). On evidence from this outbreak, it has been calculated (M.G. Underdown, 1992, personal communication) that in the absence of control agents, sirex had the potential to cause an average loss of timber from the total pine plantations in Australia valued at between US$16 and US$60 million per year. In Brazil, where 350,000 ha of pine are currently infested by sirex, it is estimated that, on an average, US$6.6 million would have been lost each year had companies not adopted an integrated pest management (IPM) programme based mainly on nematode release.

21.2. Biology of Sirex

Sirex usually has a 1-year life cycle, with adults emerging from late December to March in Australia, but as early as October with peak emergence from November to December in Brazil (Iede *et al.*, 1998), and living for about 1 week. Female sirex drill 10–20 mm into the living pine tree and insert toxic mucus and spores of a pathogenic fungus, *Amylostereum areolatum* (Coutts, 1969a,b; Gaut, 1969). If the tree is suitable,

Fig. 21.1. An area of the 50,000-ha 'Green Triangle' in southern Australia, where up to 80% of trees were killed over 2 years by sirex.

one or more eggs are also laid nearby. Depending upon the size of the female, sirex oviposit from 30 to 450 eggs (Madden, 1974). The toxic mucus prevents sugar from being passed down from the leaves. Normally sugar is converted by the tree into fungal poisons (polyphenols) at the site of fungal infection, thus stopping the fungus spreading, but this cannot happen after a successful sirex attack.

Fungal spores germinate and can now grow into the wood; within a few weeks/months a successfully attacked tree will die as a result of the mucus and fungus combined. The fungus then grows throughout the dead tree while the eggs laid by sirex hatch and the resulting sirex grubs bore into the wood, feeding on the growing fungus.

21.3. Biology of the Nematode

Only *B. siricidicola*, of seven species of *Beddingia* (Bedding, 1968, 1975; Akhurst 1975) parasitizing 31 siricid and parasitoid hosts from 31 tree species and 29 countries (Bedding and Akhurst, 1978), was found to be suitable for the control of sirex (Bedding, 1984). This nematode can achieve levels of parasitism approaching 100% when the density of sirex-infested trees is high. It has an unusual and complicated biology (Bedding, 1967, 1972, 1993) that has been exploited for the biocontrol of sirex (Bedding and Akhurst, 1974; Bedding, 1979, 1984, 1993; Iede *et al.*, 1998).

B. siricidicola is extraordinary in having two separate life cycles associated with two morphologically very different adult female types (Bedding, 1967, 1968; Fig. 21.2). There is a parasitic cycle in which from 1 to 100, 0.5- up to 2.5-cm-long, cylindrical, often green-coloured females release thousands of juvenile nematodes into the body cavity of adult sirex wasps, and a free-living cycle where 1- to 2-mm-long females feed on the symbiotic fungus as it grows in the tree and lay eggs in the wood fibres (tracheids) (Fig. 21.3). These two types of females are so morphologically different

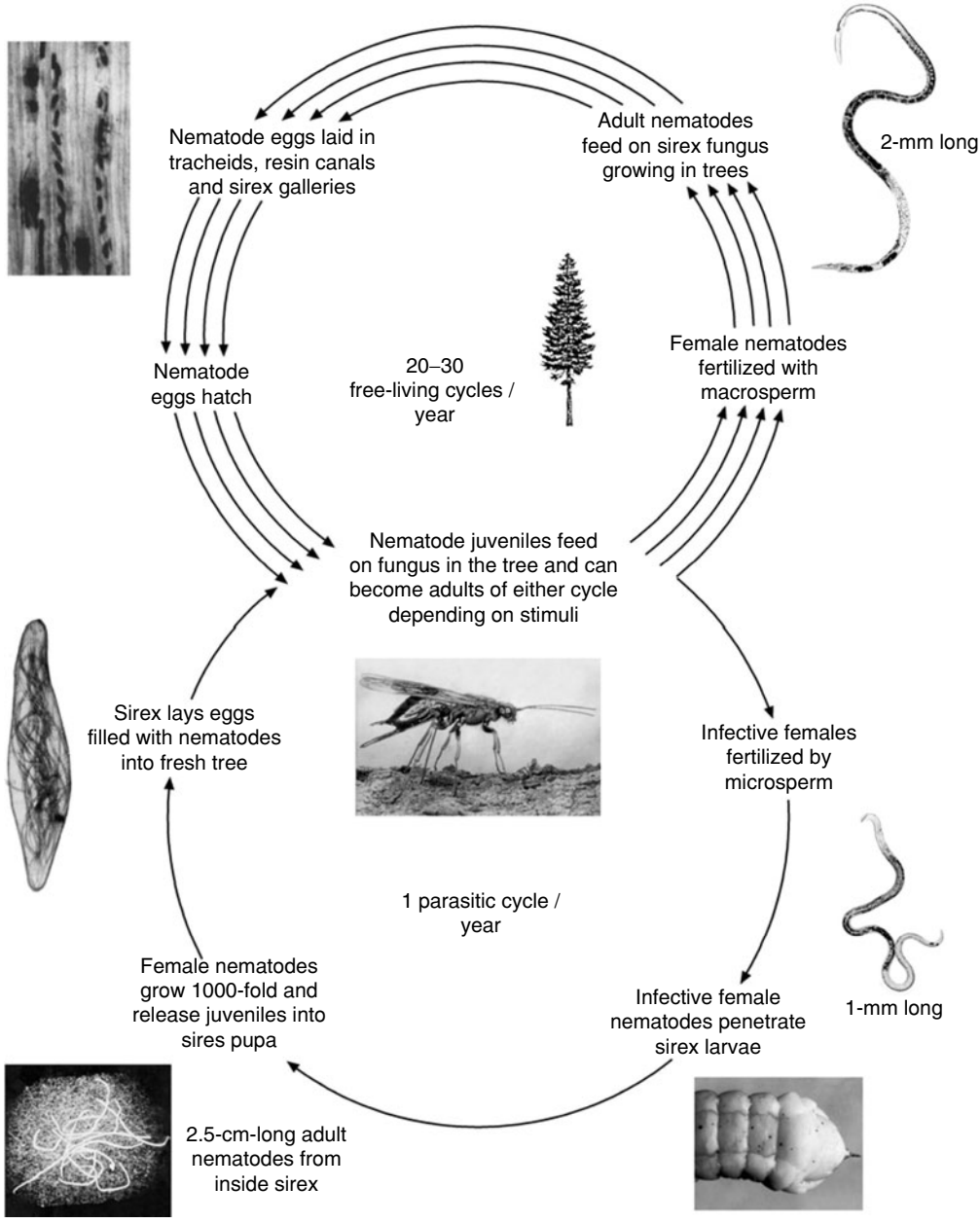

Fig. 21.2. Biology of the nematode parasite of sirex, *Beddingia siricidicola*.

that each on its own would have been placed in a separate family of nematodes.

At about the time adult parasitized sirex emerge from infested trees, adult nematodes have usually released most of the juveniles that are within them into the insect's blood cavity and the juveniles have migrated to the insect's reproductive organs. In the male sirex the testes become greatly enlarged, often fused and filled with thousands of juveniles. However, this is a dead end for the nematodes because by this time the testes

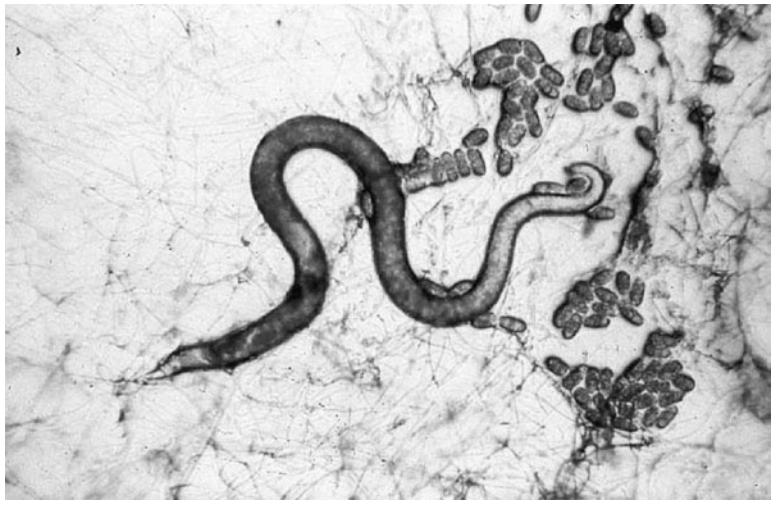

Fig. 21.3. Fungal-feeding female of *Beddingia siricidicola* with eggs on potato dextrose agar (PDA) plate.

have already emptied sperm, but not nematodes, into the insect's seminal vesicles so that parasitized males can still fertilize females but not transmit nematodes. On the other hand, female sirex are effectively sterilized because, apart from ovarial development being suppressed to various degrees, each egg that is produced is filled with up to 200 juvenile nematodes. Nevertheless, the parasitized female sirex still oviposits readily, and often in several different trees, but lays packets of nematodes instead of viable eggs. Since many sirex often attack the same trees, larval progeny of unparasitized sirex can eventually become infected with nematodes, but this is only made possible by the intervening free-living cycle.

A large tree may contain many hundreds of millions of tracheids (hollow fibres) weighing from 1000 kg to 5000 kg but rarely more than a few hundred sirex larvae. Nematodes have to migrate up and down the tracheids and through the infrequent holes in these (degenerate bordered pits) to move from tracheid to tracheid. Each egg from a nematode-infected female sirex contains not more than 200, 0.5-mm-long juvenile nematodes and the chances of many of these nematodes reaching and penetrating sirex larvae would be slim indeed, except that *B. siricidicola* breeds in vast numbers (possibly hundreds of millions) during many generations and spreads to all parts of the tree while feeding on the sirex symbiotic fungus as it grows in the tree.

It is only when larval nematodes reach the immediate microenvironment around sirex larvae that they are stimulated by high CO_2 and low pH (Bedding, 1993) to develop into the pre-parasitic kind of female rather than the fungal-feeding form. This infective female mates with special males, formed under the same conditions, that produce microspermatozoa instead of the amoeboid spermatozoa produced by normal males. After mating the infective female uses its large anterior spear-like stylet to bore into sirex larvae, after which it migrates in the insect's blood cavity for a few days before shedding its cuticle to leave its entire body surface covered with microvilli (Riding, 1970), which rapidly absorb food. Within a few weeks of penetrating a sirex larva the nematode grows up to 1000-fold in volume, depending on the size of its host. It now remains, often for several months, relatively unchanged, until the sirex larva pupates. The nematode's reproductive system then develops rapidly from a few cells to produce many thousands of juvenile nematodes in little more than a

week, and these are released into the insect's blood cavity at about the time the insect emerges from the tree. It is of interest to note that in some other species of host and with some other strains and species of the nematode, timing of juvenile nematode release can be later so that the host's eggs' shells have already formed and nematodes cannot enter the eggs. In these cases nematodes are deposited on the outside of eggs and they can then later infect larvae derived from their own host, which is essential where the siricid is a solitary species.

21.4. Application

21.4.1. General

Unlike most of the nematodes featured in this book, *B. siricidicola* is used as a classical biocontrol agent. Once it has been introduced, most of its dispersal is by females of the pest insect, sirex. However, human intervention has been and is still required to monitor and assist its spread, particularly to new sirex-infested plantations and also within them. In order to introduce the nematodes into new areas it has been necessary to isolate the best strain and develop the methods for rearing, storing, formulation, inoculation and distribution, as described below. In Australia, there is a National Strategy document (Haugen *et al.*, 1990), detailed standard operating procedures for rearing, storing, formulation and quality control (Calder and Bedding, 2002) and operations worksheets (National Sirex Coordination Committee, 2000) covering various aspects of sirex control including inoculation and distribution of nematodes. In South America, particularly in Brazil, there is a similar provision of technical and other publications (Iede *et al.*, 1998).

21.4.2. Choice of species and strain

Several hundred isolates of seven species of *Beddingia* were screened for potential to control sirex during the early 1970s. Most siricids are associated with *Amylostereum chaillettii* and this is also the only fungus on which five species of *Beddingia* can feed (Bedding and Akhurst, 1978). *B. wilsoni* fed on *A. chaillettii* and *A. areolatum* but frequently parasitized the insect parasitoids *Rhyssa* spp. and so this left only strains of *B. siricidicola* for further consideration. Many strains of this species parasitized but did not fully sterilize Australian *S. noctilio* (Bedding, 1972) and were eliminated.

Then, hundreds of randomly selected sirex-infested logs were inoculated with the various remaining strains of *B. siricidicola*. Four of these nematode strains from Corsica, Thasos, Sopron and New Zealand parasitized nearly 100% of the emerging sirex (R.A. Bedding and R.J. Akhurst, 1971, unpublished data), but it was found that sirex parasitized by the 198 strain from Sopron, Hungary, were significantly larger than those parasitized by other species. Using flight mills it was found that while parasitism itself had no significant effect on the flying abilities of sirex, insect size, which was often affected by parasitism, had a major effect. Whereas very large sirex females could fly up to 200 km on the flight mills, very small females could fly only about 2 km. Not only could large infested sirex fly further and probably infest many more trees, they also produce more eggs and more nematodes. As a result of these findings most of the releases in Australia have been of the 198 strain, and this is the only strain that has been supplied to South America and South Africa.

21.4.3. Even the best strain deteriorates

Bedding and Akhurst (1974) showed that using their methods (described below), sirex emerging from correctly inoculated sirex-infested trees were over 98% parasitized by nematodes. However, during the late 1980s, after the Victorian Forest Commission had been inoculating sirex-infested trees for over 15 years, it was found (as a result of the Green Triangle outbreak) that sirex emerging from these trees were only

about 25% parasitized (M.G. Underdown, 1992, personal communication). In Brazil the defective strain was used for inoculations from 1990 to 1994. This obviously had implications not only for the current release programme but even more importantly for the effectiveness of sirex control as a whole. The possibilities were either that incorrect inoculation procedures had been gradually adopted or that there had been genetic change in the nematode used. It was soon determined that declining parasitism in inoculated logs over several years was certainly a result of genetic change in the nematode used. While use of the fungal-feeding cycle to maintain cultures and mass-produced *B. siricidicola* is an essential part in the use of this nematode for biocontrol, it also resulted in this major problem (Bedding, 1992).

Because *Beddingia* had been cultured in the free-living form for over 20 years without intervention of the parasitic life cycle, this led to the selection of a strain that rarely formed the pre-parasitic infective stage. Even at high concentrations of CO_2 and low pH such cultures will rarely produce infective females. While there is little or no selection against a predisposition to develop into infective females in the field, the opposite is true when *B. siricidicola* is cultured artificially. The nematodes pass through repeated generations without intervention of a parasitic cycle (stock cultures had been through hundreds of generations). Infective female nematodes produced in these cultures could not reproduce because there was no insect host and so there was a strong selection pressure against their production.

The production of low levels of parasitism in inoculated logs was unfortunate and costly (four times as many trees needed to be inoculated), but of far greater significance was what this meant in terms of the ability of this 'defective' strain of nematodes to control sirex populations in plantations. There was reason to believe that nematode control with the defective strain would not occur until sirex infestations were severe (perhaps $>$ 10% tree death), whereas the original strain produced high levels of parasitism at very much lower tree death (probably $<$ 1% tree death). Results from New South Wales and South Australia tended to confirm this (R.H. Eldridge, New South Wales, 2000; M.G. Underdown, 1996, personal communication). Although the defective strain was used very effectively in the Green Triangle it was almost certainly only effective there because of the very high density of sirex infestation (up to 80% tree death) and intense nematode inoculation (20% of all infested trees).

21.4.4. Re-isolation of the nematode from area of original release

It was obviously important to obtain non-defective nematodes and this was achieved in 1991 by collecting sirex-infested timber from the Kamona forest in Scottsdale, Tasmania, where it was first liberated in 1970 but where no subsequent liberations had taken place (Bashford, 1991, personal communication). Nematodes were found and extracted from just one of the only nine sirex-infested trees found and then established in monoxenic culture on *A. areolatum* as described below. After a series of test inoculations in sirex-infested billets, it was found that this new culture, called the 'Kamona' strain, produced over 95% parasitism in the sirex that subsequently emerged compared with the defective strain where emerging sirex were only 23% parasitized. Since then the Kamona strain has been used for all liberations in Australia, South America and South Africa.

In spite of the success of the Kamona strain, there are still two major problems: (i) large areas of parts of southeast Australia were inoculated, over many years, with the 198 strain as it became progressively defective; and (ii) even the Kamona strain had several years of only fungal culturing before liberation, and we have recently found (R.A. Bedding and J. Calder, 2001, unpublished data) that it starts to become defective when subcultured in the laboratory for as little as 6 months. The first issue, to be discussed later, is being addressed by endeavouring to swamp the defective strain

already in the field with the Kamona strain. In the second case, Bedding (1993) developed methods for storing *B. siricidicola* in liquid nitrogen and stored hundreds of ampules of the Kamona strain in Dewars so that at the beginning of each season, it has been possible to begin fresh fungal cultures of the nematodes and then mass-rear them before any decline in infectivity. In Brazil, the original strain introduced in 1989 from Australia became defective and the Kamona strain was introduced in 1995; nematodes have since been re-isolated from the field every year.

21.4.5. Storage in liquid nitrogen

Although entomopathogenic nematodes (EPNs) can be readily stored in liquid nitrogen using methods developed by Popiel *et al.* (1988), Popiel and Vasquez (1991) and Curran *et al.* (1992), these methods resulted in 100% mortality of *B. siricidicola*. However, when various larval stages of *B. siricidicola* are sterilely suspended in 5% glycerol solution and water is slowly evaporated in a laminar flow cabinet, to achieve 50% glycerol after several days, the nematodes can be successfully frozen in liquid nitrogen. Vials, each containing 300 μl of the suspension, are plunged directly into liquid nitrogen, and even after 10 years there has been over 75% survival with every prospect that the remaining nematodes will survive indefinitely. The nematodes are revived by exposing a vial to running lukewarm water to ensure rapid thawing, adding and mixing 500 μl of sterile water and then placing 100-μl aliquots along the fungal front of 5-day-old cultures of *A. areolatum* growing on one-third strength potato dextrose agar (PDA) plates.

21.5. Nematode Culture

Culture of *B. siricidicola* relies upon the fungal-feeding cycle of this nematode. Because it is released inoculatively, unlike EPNs, only relatively moderate numbers of *B. siricidicola* are required even for millions of hectares of pine forest, so large-scale rearing methods have not been required. Cultures are originally established and subcultured monoxenically on PDA plates, which are then used to inoculate 500-ml flasks containing autoclaved grain. All procedures in culturing are conducted under fully sterile conditions (using sterilized equipment and media in a laminar flow cabinet with the operator wearing sterilized gloves and spraying inside the cabinet and all objects put into it with 70% ethanol).

A complicating factor when culturing *Beddingia* sp. is that the nematodes feed well only on the advancing front of the fungus; if the fungus grows too rapidly it smothers the nematode and the culture is lost; if there are too many nematodes the fungus may be unable to grow adequately.

21.5.1. Establishment of cultures of the fungus

Cultures of the fungus *A. areolatum* alone are required when nematode cultures are to be established either from liquid nitrogen or from parasitized sirex and are usually made by subculturing from already established cultures. Initially, cultures are made from live sirex females by plunging them into 100% ethanol within a laminar flow cabinet, igniting the insect and then plunging it into a Petri dish of sterile ringers in which it is dissected using sterile instruments and a dissecting microscope sprayed with 70% alcohol. The two ooidial glands found inside the insect at the base of the ovipositor are removed and streaked on PDA plates, and 4 or 5 days later areas of uncontaminated fungus are removed and placed on fresh plates.

21.5.2. Establishment of the nematode cultures from sirex

Most culturing, after initial removal from liquid nitrogen, is directly from monoxenic plates to fresh plates or, for mass rearing, to flasks. However, the initial cultures had to

Fig. 21.4. Male reproductive organs from parasitized sirex (right) and unparasitized sirex (left). It is convenient to use the infected testes to establish monoxenic cultures of *Beddingia siricidicola* on the symbiotic fungus *Amylostereum areolatum*.

be made from live parasitized sirex adults; this is still necessary when nematodes are removed from field insects to test for the infectivity and/or strain of nematodes involved using molecular biology (Fig. 21.4).

In the same way as ooidial glands were removed sterilely from the female, testes filled with juvenile nematodes are removed from male sirex and placed centrally on 5-day-old fungal cultures growing on one-third strength PDA plates.

21.5.3. Mass culture and dispatch

Stoppered, 500-ml conical flasks, each containing 90 g of an equal mixture of brown rice and wheat and 150 ml of tap water, are autoclaved for 30 min at 121°C, cooled rapidly and each inoculated by sterile spatula with about one-half the growing front from a mature (about 2-week-old) culture plate of nematodes (Fig. 21.5). Flasks are then kept at 23°C until mature (5–8 weeks) when they are harvested with water, washed, sieved and dispatched in batches of 1 or 5 million in breathable plastic bags together with

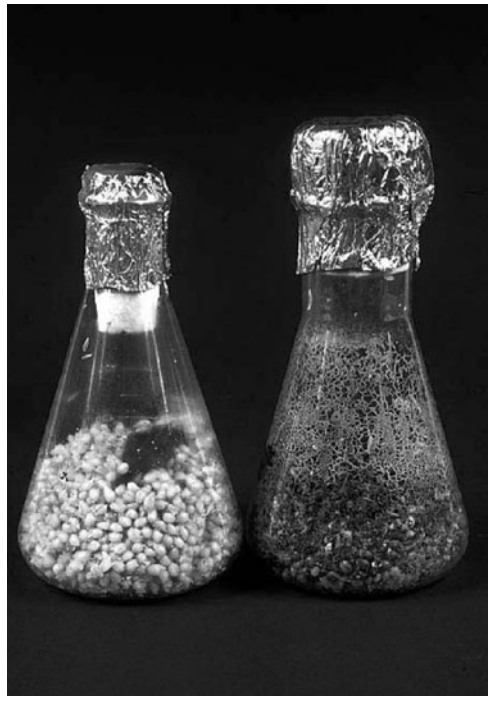

Fig. 21.5. Culture flasks just after inoculation with nematode/fungus culture (left) and just before harvest (right).

packets of finely ground (< 600 μm) polyacrylamide gel (5 g for each million nematodes).

21.6. Nematode Application

21.6.1. Plantation inoculation

In Australia, inoculation is conducted as part of a national strategy of sirex awareness, quarantine, detection, monitoring and silvicultural control (Haugen *et al.*, 1990) and this is similar in South America (Iede *et al.*, 2000).

When sirex populations are moderate (1–3%) or severe (> 3% infested) it might be necessary to inoculate 20% (every fifth row) of infested trees to obtain rapid control. However, if pre-emptive action is taken it is usually only necessary to inoculate a small number of easily accessible trap trees for each 20–30 ha of forest to be protected. Generally speaking, pine trees do not become susceptible to sirex until they are about 12 years old, and this is when action should be taken to protect such forest compartments using nematodes when sirex infestations are in the vicinity (as determined by aerial and ground surveys and/or results from trap tree plots).

To introduce nematodes, trap tree plots are established near the roadside of each 20- to 30-ha compartment where, by injecting at the base of five trees just enough weedicide (e.g. DiCamba) to almost kill them, they become highly susceptible to sirex (Madden and Irvine, 1971; Neumann *et al.*, 1982, 1989). This is conducted 2–3 months before the expected flight season and trees that become infested with sirex can then be inoculated with nematodes during April to August. It is particularly important that nematode inoculations are as well dispersed as possible throughout plantations since most nematode dispersal occurs only to the infested trees nearby; Bedding and Akhurst (1973) liberated 50 parasitized sirex at the corner of a 1000-ha plantation during 1970 and found that while nematode parasitism in the 30-ha compartment of liberation rose from 31% in 1970, 47% in 1971 to 92% in 1972, in the whole forest it had reached only 37% during those 3 years, with 6% in the first year and 14% in the second.

21.6.2. Tree inoculation

How sirex-infested trees are inoculated is of the utmost importance if most of the emerging sirex are to be parasitized without size being adversely affected (Bedding and Akhurst, 1974). During initial experiments, holes were drilled into sirex-infested billets and a suspension of nematodes was added to each hole; this resulted in negligible parasitism in the emerging sirex. The drilling resulted in the cut ends of the tracheids (wood tubes) being twisted and water was rapidly absorbed into the wood, leaving the nematodes 'high and dry'. However, when tracheids are cut cleanly, nematodes are added in a gel suspension, the wood is moist enough and the spacing of inoculation is optimum, nearly 100% of emerging sirex are parasitized.

Inoculation is currently achieved using a specifically designed, frequently sharpened, rebound, hammer punch (Fig. 21.6) that cuts the tracheids cleanly and nematodes suspended in a 1% finely ground (< 600 μm) polyacrylamide gel (Australia), or as per Bedding and Akhurst (1978) in foamed, 10% gelatine solution (Brazil). Sirex-infested trees are felled and the branches trimmed off. Inoculation holes approximately 10 mm deep are made every 30 cm with one row where tree diameter is less than 15 cm and two rows of staggered holes where tree diameter is greater than 15 cm. One million nematodes are mixed in 500 ml of 1% gel (enough for 10–20 trees) and dispensed from a sealant gun, syringe or sauce bottle so that 2000 nematodes are added per inoculation hole in about 1 ml gel. The gel is further pressed into the hole using a finger. Two operators are usually involved with one making holes and the other dispensing the gel containing the nematodes.

Fig. 21.6. Wad punch mounted in a hammer enables clean cutting of the tree's tracheids so that nematodes can enter.

21.6.3. Monitoring

Even after nematodes have been liberated in an area it is important to ensure that they are established. The main method of assessing nematode levels is to dissect sirex emerging from caged logs. The presence of nematodes can also be detected soon after trees have died by cutting chips from sirex-infested trees and standing these in shallow water for 24 h. Where there is moderate to severe sirex infestation and there is no nematode parasitism it is recommended that 20% of sirex-infested trees be inoculated; where 1–5% of sirex are parasitized, 10% of trees should be inoculated; where 5–10%, 5% should be inoculated while those with greater than 10% parasitism, no further inoculation is worthwhile.

21.6.4. Replacing defective strain

As described above, some areas of Australia and Brazil may have the defective strain of *B. siricidicola* present in sirex populations. R.A. Bedding and J. Calder (2000, unpublished data) have found that unfortunately it requires several back-crosses between Kamona and defective strains before crosses become fully infective and this is reflected in randomly amplified polymorphic DNAs (RAPDs) of the back-crosses (see Fig. 21.7). This has meant that in such areas, the Kamona strain has had to be repeatedly re-introduced until it dominates. This is achieved much more readily when levels of sirex infestation are low. Use of RAPDs to distinguish between Kamona and defective strains has proved invaluable to determine what strains dominate in the field. J. Calder (1998, unpublished data) found that out of 100 PCR primers tested, the two strains (and also various strains of EPNs) could be readily separated using the primers OP-AO4, OP-X11 and OP-FO3. In addition, the ability to form infective females can be tested by sterilely harvesting eggs from the mycetophagous cultures of *B. siricidicola* and placing these on *A. areolatum* growing on 0.2% lactic acid/PDA plates inside desiccators containing 10% CO_2.

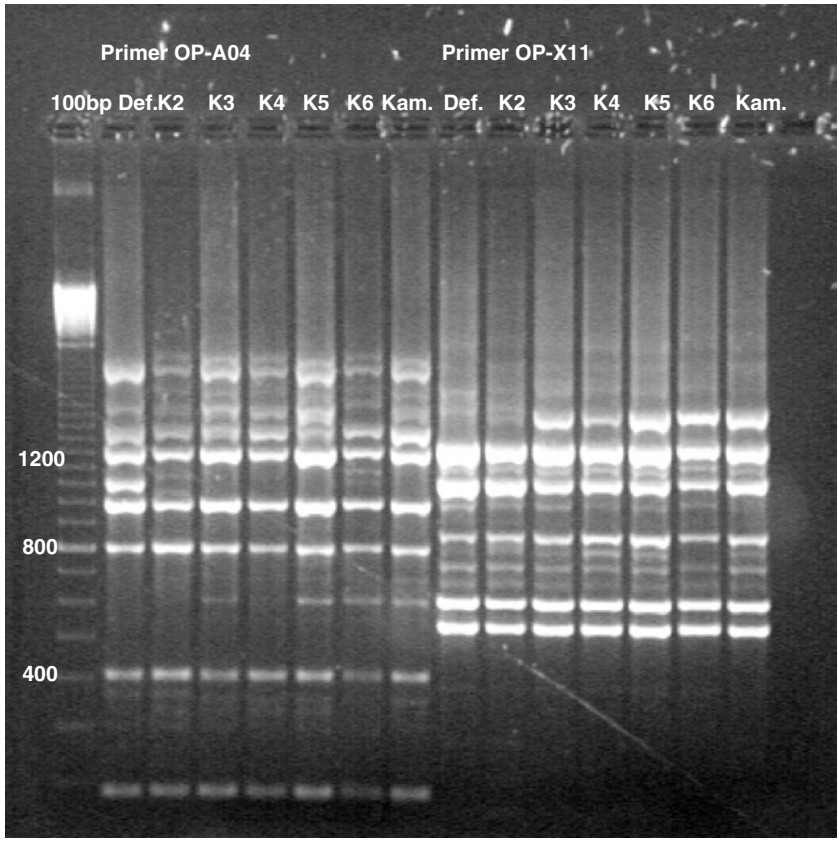

Fig. 21.7. Randomly amplified polymorphic DNAs (RAPDs) using two primers to distinguish between the 'defective' and Kamona strains derived from the original *Beddlingia siricidicola* isolate from Sopron, Hungary, and also a series of back-crosses between these two strains.

21.7. Evidence of Control

It is believed that nematode parasitism is density dependent and that provided nematodes are adequately distributed and established, control occurs before there are high numbers of tree deaths provided non-defective nematodes are in place (Bedding, 1993). During 1972, in a 400-ha forest of *P. radiata* in northern Tasmania where about 5–10% of trees had been killed annually by sirex over several years, all infested trees from every 10th row were inoculated with nematodes. In 1973, based on sampling 64 widely dispersed infested trees, parasitism was 86%, while in the following year no sirex-killed trees could be located by ground and aerial surveys. Similarly, in the Green Triangle, from 1988 to 1989 nearly 100% parasitism resulted after inoculating sirex-infested trees in one row out of every five, and ever since it has been difficult to find any sirex-infested trees. In a

12,000-ha plantation in Encruzilhado do Sul in Brazil, where sirex infested about 30% of trees in some compartments in 1991, nematodes were released from 1990 to 1993, resulting in levels of parasitism of 45% in 1991, 75% in 1992 and more than 90% in 1994. In 1995 it was difficult to find any sirex-infested trees in this area. Generally, in Brazil, where parasitism is evaluated annually in seven localities, parasitism ranged from 17% in one locality, 39%, 57% and 65% in three others to over 92% in another three localities. This sort of variation also occurs annually in the numerous sites examined in Australia, with higher levels of parasitism apparently related to higher density of sirex-infested trees (R.H. Eldridge, 2000; Underdown, 1996, personal communication). A major factor contributing to the successful control of sirex is that, although at the beginning of an infestation of a plantation there are usually plenty of suppressed or otherwise susceptible trees that are readily killed by one or a few sirex females, as these trees are utilized by sirex, and as only the more resistant trees remain, it takes more and more sirex to kill each tree.

21.8. Discussion and Conclusions

Nematode control of sirex commenced in the early 1970s and was the first commercial use of nematodes to control any pest. In terms of the value of crop saved by nematode control, it is currently undoubtedly worth more than all other uses of nematodes. However, because it is a classical biocontrol agent usually requiring only an initial inoculation into plantations, sales of the nematode amount to less than about US$40,000 per year. While this is an example of one of the most successful biocontrol projects of its kind, there is no room for complacency, even though this may be understandable since major infestations of sirex may only occur at intervals of many years, even in the absence of nematodes. There is always the possibility of nematode strain deterioration, inadequate distribution of nematodes throughout each plantation and major calamities (fire, wind and hail damage or inadequate or untimely thinning) within forests, leading to massive build-up of sirex populations before nematodes can exert control. It is also possible that strains of sirex could develop where nematodes are released too late into the pupal haemolymph to penetrate eggs because shells have already formed (as already occurs in some other host species and even other strains of *S. noctilio*). This was suspected of an isolated New Zealand population of sirex, but it turned out to be a change in the nematode strain that was responsible (R.A. Bedding, 1990, unpublished data).

Change in the nematode strain so that it becomes defective in infectivity is one of the most important problems. Annual use of liquid nitrogen-stored material should adequately deal with this problem, but whether continual re-isolation from the field followed by many fungal-feeding generations for mass production in the laboratory is satisfactory is a matter for conjecture. Currently, it is still necessary in Australia and perhaps Brazil to swamp defective strain nematodes that are already in the field, and it is now possible to use genetic probes to test for defectiveness in field-collected isolates.

Hopefully, if the situation is continually monitored, *B. siricidicola* can be used for hundreds of years to control this very serious pest that may spread to many other areas of the world.

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