Chapter 12
Controlling the Pine-Killing Woodwasp, *Sirex noctilio*, with Nematodes

Robin A. Bedding

Abstract The pine-killing woodwasp *Sirex noctilio*, a native to Eurasia/Morocco, was accidentally introduced into various Southern Hemisphere countries during the last century and has recently (2005) been detected in north-eastern North America. The parasitic nematode *Beddingia siricidicola* is by far the most important control agent of sirex and has been introduced into each Southern Hemisphere country soon after sirex became established. The nematode has a complex life cycle with morphologically very different forms. One form feeds on the tree-pathogenic, sirex-symbiotic fungus (*Amylostereum areolatum*) as this fungus grows throughout the tree, while the other form grows in and then sterilises adult female *S. noctilio*. The fungal-feeding form of *B. siricidicola* is used to mass-produce the nematode. Methods are described for liberating nematodes in pine plantations. The nematode has caused major crashes in *S. noctilio* populations so that sirex-infested trees can no longer be found in many plantations. A problem arose when it was discovered that long-term *in vitro* culture using only the fungal cycle without intervention of parasitic cycles had selected, over many years, for a nematode strain (the "defective strain") that rarely formed the infective stage and was therefore much less effective in the field. Isolation of the "Kamona strain", annual replenishment from liquid nitrogen storage and other procedural changes are enabling strain replacement in the field. While nematode control in most of the Southern Hemisphere has proved to be highly successful, there are problems in the KwaZulu-Natal region of South Africa where warm dry winters cause sirex-infested trees to dry out before the nematode populations can spread throughout the tree. In North America an inferior strain of nematode appears to have been accidentally introduced with sirex. The symbiotic fungus of sirex introduced to North America is a different strain of *A. amylostereum* to that in the Southern Hemisphere and does not permit optimal nematode development.

R.A. Bedding
CSIRO Entomology, P.O. Box 1700, Canberra ACT 2601 Australia
e-mail: robin.bedding@csiro.au

A.E. Hajek et al. (eds.), *Use of Microbes for Control and Eradication of Invasive Arthropods*,

12.1 Introduction

Interest in nematode control of *Sirex noctilio* (sirex) has increased greatly since this severe pest of pine (*Pinus* spp.) trees spread north in South Africa into KwaZulu-Natal in 2002 (Dyer 2007) and was found in northern USA in 2004 (Hoebeke *et al*. 2005) and southern Canada during 2005 (de Groot 2007).

Sirex noctilio is the only siricid out of about 40 siricid species infesting conifers world-wide that can kill relatively healthy pine trees. Endemic to Eurasia/Morocco, where it does little harm to native pines (Spadberry & Kirk 1978), *S. noctilio* has become a major pest in the Southern Hemisphere. It was first detected in New Zealand (1940s), then Tasmania (1952), mainland Australia (1961), Uruguay (1980), Argentina (1985), Brazil (1988), South Africa (1994) and Chile (2001). In some of the worst affected areas sirex has resulted in up to 80% tree death.

In the whole of the Southern Hemisphere there are about 7.6 million hectares of commercial pine plantations currently threatened by *S. noctilio* (Iede *et al*. 2000, Wood *et al*. 2001) with potential damage estimated at US$16 million–US$60 million per annum for Australia's 1 million ha of pine (Bedding & Iede 2005) and US$23.2 million per annum for Brazil's 1.8 million ha (Iede *et al*. 2007).

The recent establishment of *S. noctilio* in North America represents a huge increase in potential damage with 58 million hectares of susceptible forests in the USA (Schneeberger 2007) and over 142 million hectares in Canada (CanFI2001 Database 2007). Using the computer program CLIMEX (Sutherst *et al*. 1999), Carnegie *et al*. (2006) show that sirex could become established even more widely after accidental introduction to additional countries, including in Paraguay, Bolivia, Peru, Ecuador, Columbia and Venezuela in South America, most of Mexico, USA and Canada in North America, and Guatemala, Costa Rica and Panama in Central America. In Africa they showed that in addition to South Africa, pine forests in Zimbabwe, Mozambique, Madagascar, Tanzania, Uganda, Kenya and Ethiopia were climatically suitable for sirex. In China, pine forests from Yunnan Province in south-central China through most provinces to Heilongjiang Province in northeastern China would be climatically suitable for the establishment of sirex.

Forest hygiene and particularly timely thinning has an important impact on sirex populations. However, while several insect parasitoids have been introduced into the Southern Hemisphere, these, perhaps with the exception of the endoparasite, *Ibalia leucospoides*, have limited effect. Of seven species of the nematode genus *Beddingia*[1] (= *Deladenus*) (Bedding 1968, 1975) found parasitising siricids and their parasitoids (Bedding & Akhurst 1978), only *B. siricidicola* was found to be suitable for the control of sirex (Bedding 1984). This nematode has now been released and established in Australia, New Zealand, Brazil, Uruguay, Argentina, Chile and South Africa. The nematode is strongly density dependent, can achieve levels

[1] Those species previously included in the genus *Deladenus* having both free-living and parasitic life cycles associated with extreme adult female dimorphism, were assigned to a new genus, *Beddingia* by Blinova and Korenchenko (1986). This nomenclature was adopted by Remillet and Laumond (1991) and by Poinar *et al*. (2002) (who also established a new family, Beddingiidae with *Beddingia* as its only genus).

of parasitism approaching 100% and is generally recognised as the main controlling agent of sirex in the Southern Hemisphere (Iede *et al*. 2000, Carnegie *et al*. 2005, Bedding & Iede 2005).

12.2 Pest Biology

Generally sirex attack only pine plantations that are over 10–12 years old. In the early stages of infestation, female sirex are attracted to suppressed, drought-stressed or damaged pine trees (Madden 1977). As the sirex population builds up over several years and there are increasing numbers of sirex available to attack each tree, more and more vigorous trees can also be killed.

Sirex kills pine trees by injecting a toxic mucous (Coutts 1969a, b) and spores of a tree pathogenic fungus *Amylostereum areolatum* (Gaut 1969) 10–20 mm deep into the wood (see Fig. 12.1). If the sirex female detects that the tree is suitable, she will lay one or more eggs in adjacent drills. Depending on size, a female sirex can oviposit from 30 to 450 eggs (Madden 1974) and may oviposit in several different trees (JL Madden personal communication).

The injected mucous suppresses translocation of sugars, and as a result, formation of polyphenols at the site of oviposition that would normally suppress fungal growth. Depending on the resistance of the tree, the fungus begins to grow significantly a few weeks to several months after oviposition and the local drying out of the wood resulting from the fungal growth causes the sirex eggs to hatch (Madden 1968). At about this stage, the tree dies and subsequently the fungus spreads throughout the tree. The sirex larvae bore through the tree feeding on the symbiotic fungus and after many months may have grown several centimetres in length before pupating near the surface. Sirex adults bore out of the wood to emerge from late December to April in mainland Australia but later in Tasmania, whereas in Brazil emergence begins in September (Iede *et al*. 1998). Usually there is one generation per year but there may be two in Brazil and one generation can take 1–3 years in Tasmania. Adult sirex live for 2–3 weeks.

Fig. 12.1 A female *Sirex noctilio* inserting toxic mucous, fungal spores and eggs into a pine tree

12.3 Nematode Biology

The nematode, *B. siricidicola*, was first discovered infecting *S. noctilio* in New Zealand, by Zondag (1962). Its life cycle and the biology of various strains in various siricid species were described in detail by Bedding (1967, 1972, 1984). The life cycle is an extraordinary one involving two very different forms of adult female, each associated with a different life cycle (Bedding 1967, 1972, 1984, 1993). One form of the nematode parasitises and sterilises female sirex, and the other form feeds on the sirex's symbiotic fungus as the fungus grows throughout the infested tree (Fig. 12.2).

12.3.1 Parasitism of Sirex

There may be 1 to more than 100 adult parasitic nematodes within the haemocoel of a single sirex; usually they are found within the abdominal cavity but may be in the thorax and even within the legs and testes. They are cylindrical in form and vary from a few mm in length up to 25 mm in rare cases; occasionally they are green in colour. Just before a sirex adult emerges from a tree, each adult parasitic nematode will have released many hundreds of juveniles into the sirex haemocoel. Most of these juvenile nematodes migrate into the testes or ovaries of their host. The testes may become greatly hypertrophied and often fused. The ovaries are usually somewhat atrophied and juvenile nematodes penetrate each egg before the shell has hardened (in most strains of sirex and nematodes). The female sirex is thus completely sterile. Parasitised sirex oviposit readily but introduce packets of nematodes into the tree instead of viable eggs. From an evolutionary standpoint this situation can only occur because *S. noctilio* is a communal insect with many sirex (some unparasitised) usually ovipositing on the same tree. In various other species of siricids that are solitary, juvenile nematodes do not penetrate the eggs but are introduced, surrounding the outside of the egg, during oviposition (Bedding 1972).

In the parasitised male sirex, spermatozoa pass from the testes into the vesiculae seminales well before juvenile nematodes invade the testes. No juvenile nematodes pass down the sirex's vas deferens into the vesiculae seminales so that nematodes cannot be transferred from male to female sirex during copulation and the male is thus a dead end for the nematode. (However, the otherwise sterile testis filled with thousands of nematodes makes an easy vehicle to establish aseptic cultures of the nematodes (see Fig. 12.3B)).

12.3.2 The Fungal Feeding Cycle

Bedding (1967) found that when juvenile nematodes were removed from parasitised sirex and placed on cultures of the sirex symbiotic fungus, *A. areolatum*, the nematodes readily fed on the growing fungus, grew into adults quite unlike the parasitic

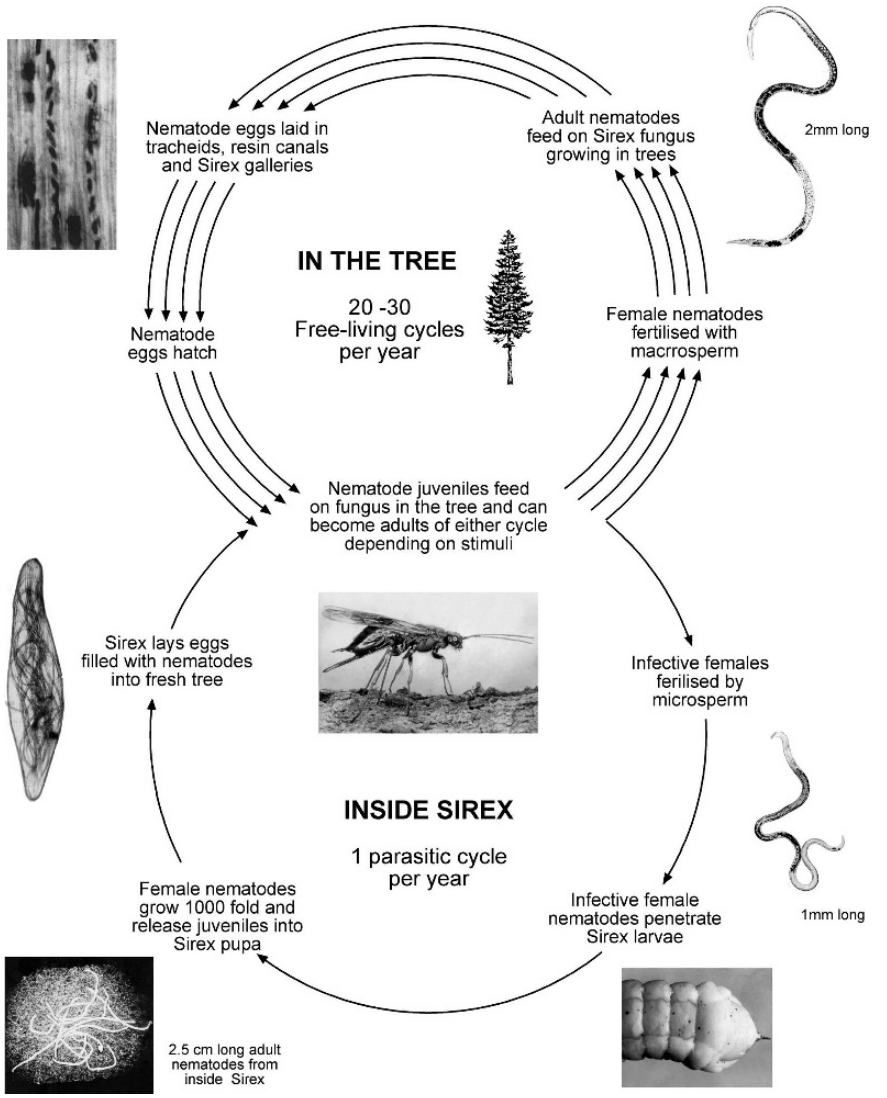

Fig. 12.2 Biology of the nematode parasite of sirex, *Beddingia siricidicola* (after Bedding 1993)

form and the female adult nematodes laid eggs (Fig. 12.3A). When the nematode eggs hatched, the resulting juveniles also fed on symbiotic fungus and grew into adult males and females that laid eggs. This cycle could be repeated indefinitely without intervention of a cycle parasitic in sirex. Similarly, when juvenile nematodes are introduced into trees by nematode-parasitised sirex, these juveniles move through the tracheids of the tree feeding on the fungus, growing into adults and breeding in vast numbers as the fungus grows throughout the tree. Without the

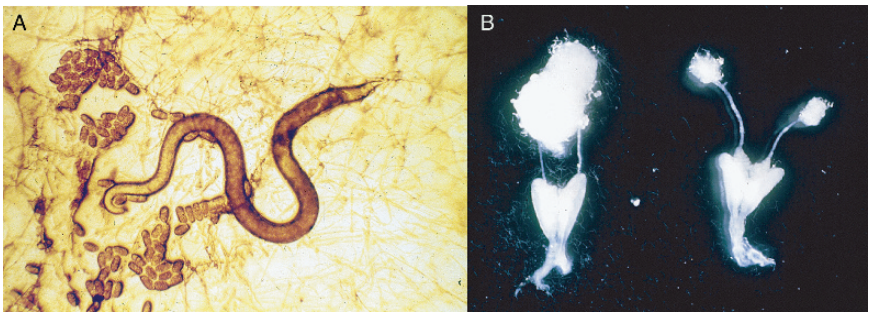

Fig. 12.3 **A**. Mycetophagous female and eggs of *B. siricidicola* on potato dextrose agar culture of *Amylostereum areolatum*. **B**. Male reproductive organs from parasitised sirex (*right*) and unparasitised sirex (*left*). Infected testes are used to establish monoxenic cultures of *Beddingia siricidicola* on the symbiotic fungus *Amylostereum areolatum*

multiplication of free-living nematodes within a sirex-infested tree, there would be, at the most, a few thousand juveniles in often hundreds of kg of wood comprised of hundreds of millions of tracheids within the tree and the chances of these juvenile nematodes reaching significant numbers of sirex larvae would be minimal. As it is, the free-living nematode population breeds throughout the tree and can reach most of the sirex larvae (progeny of unparasitised sirex) feeding within it (provided moisture levels are adequate).

Until nematode populations reach sirex larvae these free-living cycles continue, with a cycle possible every 12 days or so at 24°C (Akhurst 1975). However, when part of the nematode population is in close proximity to a sirex larva, the local microenvironment (high CO_2 and low pH) has a dramatic impact on nematode eggs and young juvenile nematodes (Bedding 1993). Instead of becoming mycetophagous, egg-laying adult females and associated males, they become infective females and their associated males.

12.3.3 The Infective Stage

The infective female is morphologically very distinct from the mycetophagous female (Bedding 1968, 1972); in fact it is so different that when discovered it would have been placed in a separate family. The extreme dimorphism reflects the differences in function of the two forms. Whereas the stylet of the mycetophagous female is like a fine hypodermic syringe, adapted to pierce and suck fluid from the fungal hyphae, the stylet of the infective female is twice as long and a much stouter spear used to puncture the cuticle of the sirex larva so that the infective female can gain entry. The very different gland structures are no doubt similarly adapted to function. The reproductive system of the infective female consists largely of a long cylindrical oviduct terminated by a few generative cells. Because only the female penetrates sirex larvae, she cannot be fertilised after entry and must take all the

spermatozoa that are required to produce thousands of progeny with her. This has required another morphological adaptation: whereas mycetophagous females (that can re-mate when necessary) are fertilised by males having giant amoeboid sperm, another kind of male producing microsperm (almost entirely nucleus) fertilises the infective female. Because of the very small size of the micro spermatozoa, the infective female oviduct can hold many thousands of them.

The infective female nematode penetrates sirex larvae, and occasionally sirex pupae, after repeatedly probing with its spear-like stylet to puncture the larval cuticle. Then it slips into the sirex larval haemocoel usually leaving a scar that remains until the next sirex larval moult (Bedding 1972). It then moves around inside its host for a few days before shedding its cuticle (not at true moult) to reveal its entire body surface covered with microvilli (Riding 1970). The microvilli are able to rapidly absorb food from the sirex larval blood and within a few weeks the infective female nematode (now a parasitic female), initially only about 0.6 mm long, can grow up to 1000 fold in volume and reach 20 mm in length. However, the reproductive system of the infective female remains more or less the same size until the onset of the host's pupation. Then, presumably as a result of the hormonal changes within its host, the few cells at the tip of the nematode's oviduct proliferate profusely and dramatically, eventually developing into many hundreds of eggs that are fertilised by the microsperm filling the nematode's oviduct.

Before the sirex female host emerges from a tree, the nematode eggs hatch and the parent nematode becomes little more than a tube filled with juveniles that now force their way out from all over the parent's surface and into the blood of the sirex host and thence to the reproductive organs. Although these juveniles appear to be identical to those hatching from eggs in the free-living cycle, when they are placed on fungal cultures and exposed to high levels of CO_2 and low pH they develop into only mycetophagous adults and never into infective females (RA Bedding & J Calder unpublished data). Presumably the same is true within the tree: when sirex introduces juvenile nematodes into a tree during oviposition, there must be at least one fungal feeding/breeding cycle before infection of a sirex larva can occur.

12.4 Manipulation of the Nematode for Control of Sirex

B. siricidicola is essentially used as a classical biological control agent; after nematodes have been introduced into a plantation, parasitised sirex females emerge from infested trees and disperse the nematodes to trees freshly attacked by unparasitised sirex. As the sirex population builds up over several years, more and more parasitised sirex attack each susceptible tree and this leads to higher and higher percentage parasitism of sirex larvae parented by unparasitised sirex ovipositing on the same tree. Finally the sirex population crashes because once the most susceptible trees have been killed it takes larger numbers of sirex than are available to kill the healthier trees. However, human intervention is necessary for continual control because nematode-parasitised sirex are usually smaller (they have had to compete

with the nematode population for fungal food) and don't fly as far as unparasitised sirex so that it is the latter that usually initiate infestations in newly susceptible plantations. (Plantations/compartments do not usually become susceptible until 10–12 years of age). As a result, nematodes must be introduced as soon as sirex is detected in a new area and until nematodes are confirmed to be established.

All is not quite so simple though since it has been important over many years to isolate the best species and strain of nematode and to develop methods for rearing, storing, formulation, inoculation, distribution and quality control.

The free-living, fungal-feeding cycles of *Beddingia* species not only significantly increase the nematodes' ability to find and parasitise their hosts in the natural state, they have greatly facilitated manipulation of these nematodes for the biological control of sirex (Bedding & Akhurst 1974, Bedding 1979, 1984). Using fungal cultures enabled the storage of a large library of cultures of different species and strains of *Beddingia* over many years and also allowed for the development of a method for mass producing the chosen nematode strain for liberation.

12.4.1 Selection of Best Species and Strain for Original Liberations

Throughout the 1960s and early 1970s, CSIRO personnel, Drs. Philip Spradberry and Alan Kirk, and various consultants conducted a comprehensive search, in hundreds of localities from Europe, USA, Canada, India, Pakistan, Turkey, Morocco and Japan, for coniferous trees infested with various siricid species. Thousands of logs from these trees were then caged in quarantine, mainly at Silwood Park, UK, where all emerging insects were investigated for biological control potential. As a result of these collections, seven species of *Beddingia* were found parasitising 19 siricids (associated with two fungal symbionts) and 12 parasitoids from 31 tree species and 29 countries (Bedding & Akhurst 1978).

Beddingia species were dissected from thousands of insects from hundreds of sources. These nematodes could not have survived for more than a few days had it not been possible to culture them on the symbiotic fungi of their hosts. As it was, hundreds of monoxenic nematode cultures were readily established on fungal cultures on potato dextrose agar (PDA) plates and slants in tubes and placed under refrigeration after initial establishment. Most cultures were sub-cultured every 6 months or so and maintained for several years during evaluation, classification and experimentation.

Out of the hundreds of nematode cultures assessed during 1970–1974 for suitability to control sirex in Australia, all cultures of five species were rejected because they parasitised siricids associated with the symbiotic fungus *Amylostereum chaillettii* and could not feed on the symbiont (*A. areolatum*) of *S. noctilio* (Bedding & Akhurst 1978). Of the two remaining species, *Beddingia wilsoni* was rejected because, although it parasitised sirex and fed on both *A. chaillettii* and *A. areolatum*, it also parasitised the beneficial rhyssine parasitoids. *B. siricidicola* remained the chosen species but it was then a matter of selecting which strain would be most suitable for development for biological control.

Several strains of *B. siricidicola* parasitised sirex but did not enter the eggs of their host (Bedding 1972) and were therefore rejected. The many remaining strains were inoculated into hundreds of randomly selected sirex-infested logs to compare rates of parasitism. Four strains (from Corsica, Thasos in Greece, Sopron in Hungary and New Zealand) were found to give nearly 100% parasitism of emerging sirex (Bedding & Iede 2005). Small numbers of each of these strains were liberated in Victoria (where sirex first established on the mainland of Australia) in the early 1970s. However, it was found that sirex parasitised by the 198 strain from Sopron (which was derived from a single parasitised female *S. juvencus*) were significantly larger than those parasitised by the other strains. The size of parasitised sirex females was important, because flight mill studies established that although nematode parasitism itself did not affect the distance flown, size had a major impact. Thus, small sirex could often fly no further than 2 km on flight mills whereas the largest sirex flew up to 200 km. Large sirex also oviposit more and survive longer and so if parasitised are much better at distributing the nematode. For these reasons all liberations (in Australia, South America and South Africa), subsequent to these findings (post 1971), were of the 198 strain.

12.4.2 Mass Rearing B. siricidicola *for Liberation*

It is neither necessary nor feasible to use the parasitic life cycle to produce the millions of nematodes required for liberation. The nematodes can be cultured readily on the symbiotic fungus, *A. areolatum*. Initial cultures are made on potato dextrose agar plates and these are then used as inoculum for 500 ml erlenmeyer flasks containing 90 g wheat or wheat/rice autoclaved in 150 ml water to produce a matrix of sterile swollen grains with air spaces between each grain (Fig. 12.4A). All procedures are conducted under aseptic conditions.

Aseptic fungus is initially isolated by dipping a living sirex female into 100% ethanol and then igniting it before plunging it under sterile water and dissecting out its ooidal glands (found at the base of the sirex's ovipositor) in a laminar flow cabinet. An ooidial gland is then streaked across a PDA plate prior to subculture. Similarly, the testes full of juvenile nematodes (Fig. 12.3B), are removed from a parasitised male sirex and then placed on a 4 day culture of the fungus on PDA.

Once monoxenic cultures are established these can then be readily subcultured onto fresh PDA plates and there should be no need to re-establish cultures from parasitised sirex again. Because the nematodes feed readily only on the growing front of the fungal culture, experience is necessary to determine when to sub-culture and how much inoculum to use; if there are too many nematodes/fungus, the fungus is unable to grow readily, whereas if there are not enough nematodes/fungus, the fungus grows too rapidly and may smother the nematodes and invade the nematode eggs.

Flasks are incubated for between 5 and 8 weeks and are harvested when most fungus has been consumed (see Fig. 12.4A). Harvesting is achieved simply by adding

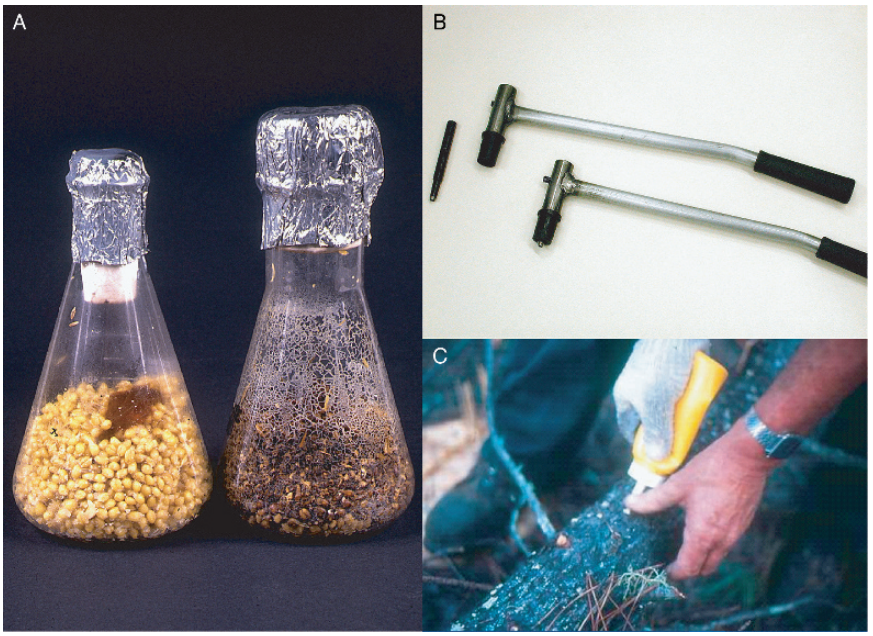

Fig. 12.4 **A**. Culture flasks just after inoculation with nematode/fungus culture (*left*) and just prior to harvest (*right*). **B**. Wad punch is mounted in a hammer to enable clean cutting of the tree's tracheids to permit nematode entry. **C**. Nematode/gel is squeezed from a sauce bottle into each inoculation hole

tap water to mature flasks to just cover the medium within, leaving for about 15 minutes with occasional gentle swirling and then decanting the resulting nematode suspension through a sieve into bowls to settle for about 20 minutes. This is repeated three times for each flask. After settling, the nematodes are washed by decanting, counted and adjusted to a concentration of 100,000 nematodes per ml. Usually 5 million nematodes are added to each breathable (e.g. Everfresh®) plastic bag; the bags are sealed and then layered between packing foam inside polystyrene boxes together with insulated freezer bricks. Boxes are consigned for next day delivery and packets of finely ground (<600 micron) polyacrylamide gel, allowing 5 g for each million nematodes, are included for final mixing. Detailed standard operating procedures for rearing, harvesting, counting, storing, formulation and quality control have been summarized by Calder & Bedding (2002).

12.4.3 Inoculation of Sirex-Infested Trees

The way in which sirex/*Amylostereum* infected trees are inoculated is particularly important. During initial experiments, holes were drilled into sirex-infested billets and a water suspension of nematodes was added to each hole but this resulted

in negligible parasitism in the emerging sirex. It was later found that the drilling resulted in twisted, blocked tracheids (wood tubes) and that the water was rapidly absorbed into the wood leaving the nematodes "high and dry". However, cleanly cutting tracheids to make inoculation holes, using an inoculum of nematodes suspended in gel, inoculating timber of adequate moisture content and using correct inoculum size and spacing resulted in nearly 100% of emerging sirex being parasitized, without the size of these sirex being adversely affected (Bedding & Akhurst 1974).

Currently, inoculation holes are made in felled sirex-infested trees, with frequently sharpened, rebound hammer wad punches (Fig. 12.4B), and the holes are injected with nematodes suspended in 1% < 600 micron, polyacrylamide gel (in Australia and South Africa) or as per Bedding & Akhurst (1974) in foamed, 12% gelatine solution (in South America). Inoculation holes are made about 10 mm deep and every 30 cm. There is one row of holes where the tree diameter is less than 15 cm and two rows of staggered holes where tree diameter is greater than 15 cm. Approximately 2000 nematodes are added per inoculation hole in about 1 ml of gel, which is pressed into the hole using a finger. Usually one operator makes the holes while another dispenses the gel/nematodes (Fig. 12.4C). The spacing of inoculations and numbers of nematodes is important: too many nematodes and/or more closely spaced inoculations result in smaller, less effectively dispersing sirex (nematodes compete with sirex larvae for fungal food) whereas too few nematodes with wider spacing results in lower levels of parasitism. It is possible that parameters may have to be adjusted for different species of host tree and/or climate with, for example, closer inoculations being better where colder climates may reduce the number of generations of mycetophagous cycles per year.

12.4.4 Liberation Strategy

Although nematodes can sometimes be spread from forest to forest by sirex females (Bedding 1979), this is too unreliable and may occur too late in the infestation of a plantation to prevent a serious outbreak. Nematodes are therefore introduced as early as possible after sirex arrives in a plantation by artificially inoculating trees already infested by sirex (as described above) at easily accessible, strategic points within a forest. In Australia and South America, inoculation with *B. siricidicola* is part of a national strategy for sirex control, also comprising quarantine, detection, monitoring and silvicultural control (Haugen *et al*. 1990, Iede *et al*. 2000) and in Australia there are also operations worksheets (National Sirex Co-ordination Committee 2002). The Australian National Strategy and worksheets are currently being updated (N Collett personal communication).

In most Southern Hemisphere countries, sirex has not yet completed its likely geographic spread (current evidence suggests an unassisted spread of about 30 km per annum) and it has only just begun to establish in North America. In addition, since sirex does not usually attack pine trees until they are about 12 years old, there are always plantations or compartments that sirex has only just reached. This situation is determined by aerial and ground surveys and by results from trap tree

plots and/or chemical lures. Trap trees (Madden & Irvine 1971) are particularly useful both for monitoring and, if found to be infested by sirex, for inoculation with nematodes. Although trap trees can be more sparsely distributed for monitoring, as a means of introducing nematodes, a trap tree plot of about five trees is established at an easily accessible spot (e.g. road side) for each 20–30 ha plantation compartment. To do this, just enough herbicide (e.g. DiCamba) to almost kill trees is injected at the base of each tree 3 months before expected sirex emergence; this makes the trees highly susceptible to sirex. Trees found to be infested with sirex are then inoculated with nematodes. Ideally this is all that is required to establish nematodes within a compartment. However, where sirex populations are already established in over 1% of trees, it may be necessary to inoculate all infested trees in every fifth row (20% of infested trees) to achieve rapid control. Obviously careful monitoring is therefore particularly important.

12.4.5 Monitoring

Even after nematodes have been liberated in an area it is important to ensure that they are established. To do this, logs collected from sirex-infested trees are caged and emerging sirex are dissected to determine levels of parasitism. Whether nematodes are present in particular sirex-infested trees can also be detected by cutting chips from along the tree and standing these in shallow water for 24 hours. Experience is required to distinguish these nematodes microscopically from other species that may be present and of course chipping cannot determine percentage parasitism of sirex within the tree. Despite these difficulties, chipping has the advantages of being much less labour consuming, it can be conducted on a greater number of trees than caging and it does not deplete the numbers of parasitised sirex emerging and transmitting nematodes to trees newly infested by sirex.

Much work remains to be conducted on exactly what are the most efficient and effective guidelines for introducing nematodes where nematode parasitism is already present but low. Current Australian guidelines are that if no nematode parasitism is detected in a compartment and more than 1% of trees are infested by sirex, 20% of these trees should be inoculated; with 1–5% sirex infected 10% of infested trees should be inoculated, and with 5–10% sirex infected 5% of infested trees should be inoculated. However, where parasitism is higher than 10%, no further inoculation is likely to be worthwhile.

12.5 Success of Nematode Control

Bedding (1993) claimed that nematode parasitism of sirex is density dependent. The density referred to is of the percentage of trees infested by sirex in a given area (but may not apply to the density of sirex larvae within a given tree). Essentially, if nematode parasitism is present in a population, the more sirex there are in a given area, the more

oviposition (by both healthy and parasitized female sirex) there will be on each tree. Hopefully this leads to high percentage parasitism and collapse of the sirex population well before a high percentage of trees are killed; this is provided that non-defective nematodes are in place and well distributed when sirex infestation is low.

Because detailed forest assessments are very labour intensive and expensive, the number of case studies has been limited to those reported by Bedding & Iede (2005). Thus, one year after inoculating all infested trees in every tenth row in a 400 ha pine plantation in Mt. Helen, Northern Tasmania, during 1972, parasitism reached 86% and in the following year, extensive ground and aerial surveys revealed no sirex-infested trees. This was despite 5–10% tree death from sirex attack during the several previous years when no nematodes were present.

Nematode treatment was conducted on a much larger scale in the 113,000 ha *Pinus radiata* plantations of the "Green Triangle" area of Victoria/South Australia where, in the absence of nematodes, a total of nearly 5 million trees were killed by sirex between 1987 and 1989 (80% tree death in some areas). Here, in 1987, in an operation costing AU$1.3 million, all infested trees in every fifth row were inoculated with nematodes (Haugen & Underdown 1990). By 1989, nearly 100% of sirex were parasitised, the population crashed and it has been difficult to find any sirex-infested trees there ever since. These results were achieved even though, as was found later, the nematodes used were of the defective strain (see below); presumably it was because of the very high density of sirex that the nematodes were effective nonetheless.

Nematodes were released from 1990 to 1993 in a 12,000 ha plantation in Encruzilhado do Sul, Brazil, where 30% of trees were infested by sirex in some compartments during 1991. This resulted in parasitism of 45% in 1991, 75% in 1992 and 90% in 1994 and it was difficult to find any sirex-infested trees in 1995. Elsewhere in Brazil, parasitism by *B. siricidicola* was evaluated annually in seven localities and ranged from 17, 39, 57 and 65% in four localities and over 92% in three other localities. Such variation also occurs in Australia and seems to be related to the density of sirex-infested trees (Bedding & Iede 2005).

In the Cape Peninsula of South Africa where sirex was first detected in 1994, 296 sirex-infested trees were inoculated in a 90 km arc around Cape Town during 1995–1996. Resulting nematode parasitism increased from 22.6% in 1996 to 54% in 1997 and to 96.1% in 1998, with never more than 3.2% tree death in any forest compartment (Tribe & Cillié 2004). Currently it is difficult to find sirex-infested trees in the Cape region.

12.6 Problems Arising in Nematode Control

12.6.1 Australia

12.6.1.1 Decline in Infectivity

The main problem that has arisen in Australia with nematode control was revealed during the Green Triangle outbreak when, instead of achieving yields of nearly

100% parasitised sirex from inoculated trees as expected from using the methods of Bedding & Akhurst (1974), only about 25% of emerging sirex were parasitised (Bedding & Iede 2005). Although this could have been the result of incorrect procedures having developed during the 15 years that the Victorian Forest Commission had been responsible for inoculations, this was found not to be the case. In fact, it transpired that there had been declining parasitism from inoculated trees over many years and that this was the result of genetic changes in the nematodes used (Bedding 1992). These changes were the result of continual sub culture of the nematodes in the fungal feeding cycle over some 20 years without the intervention of the parasitic cycle.

When fungal cultures of *B. siricidicola* are sub-cultured at an optimal stage and are kept so that there is little accumulation of CO_2 and acidity, there is little tendency for the cultures to produce infective females. However, until the problem of genetic change was discovered, culture plates were often kept for long periods in plastic bags before sub-culturing; this allowed for the accumulation of CO_2 and acidity and this initially stimulated the formation of many infective females on the plates. When these plates were sub-cultured, only those nematodes that had not responded to the CO_2 and acidity levels occurring were able to grow and reproduce, since infective females need to infect sirex to complete their cycle. In other words, there was a continual selection against the tendency to form the infective stage. Gradually this selection produced what Bedding (1993) termed the "defective strain". Unfortunately, decline of the nematodes being liberated occurred over many years and it is not known when the decline started to become important and therefore what areas became "contaminated" by inoculations with the less effective strain as sirex spread across Australia and later South America.

Although it was low levels of parasitism in inoculated logs that drew attention to the problem, of far greater significance was what that meant in terms of the ability of this defective strain of nematodes to control sirex populations once liberated. There is every reason to expect that nematode control with the defective strain may not occur until sirex infestations are severe (perhaps $>$ 10% tree death) whereas the original strain produced high levels of parasitism at very much lower tree death (probably $<$1%). The defective strain was almost certainly only effective in the "Green Triangle" because of the very high density of sirex infestation (up to 80% tree death) and intensive liberation.

12.6.1.2 Re-Isolation of Original Strain

Once the problem of the defective strain was evident, it was obviously important, but not at all easy, to replace it. To collect and select new strains from overseas would have been a huge task. Even collections from the original location in Sopron might not have yielded a suitable strain since the original strain was from a single parent nematode, was inbred for many generations and was likely to have been subjected to genetic drift before testing; it is possible that this could have led to a strain highly suitable for manipulation but one which, because it is so virulent, would not have survived indefinitely in the field. Most sirex-infested

localities within Australia had had recent introductions of defective strain so it was not possible to obtain the uncontaminated original strain except from one area. Dick Bashford from the Tasmanian Forestry Department confirmed that Kamona plantations, near Scottsdale in Northern Tasmania, had not had any nematode introductions since the very first liberations of the 198 Sopron strain in Australia in 1970. In 1991, Bashford was able to find only 9 sirex-infested trees in the whole area but one of these had nematodes. The nematodes were established in monoxenic culture on *A. areolatum*, and a series of test inoculations of sirex-infested logs produced over 95% parasitism compared to 23% with the defective strain (Bedding 1993). The new isolate also produced infective females readily on culture plates exposed to acidity and 10% CO_2. Using randomly amplified polymorphic DNAs (RAPDs), J. Calder found that three primers (OP-A04, OP-X11, OP-FO3), out of over 100 tested, could differentiate between the defective strain and the strain re-isolated from Kamona, even though both strains were derived from the original isolate from Sopron (Bedding & Iede 2005). This new strain, named the Kamona strain (Bedding 1993), has been used for inoculations in Australia since 1991 and was introduced into Brazil and South Africa in 1995.

12.6.1.3 Avoiding Infectivity Decline

Even the Kamona strain had been originally sub-cultured on fungus without intervention of the parasitic phase for several years prior to liberation and was found after re-isolation to lose some infectivity after only 6 months of sub-culture on fungus (Bedding & Iede 2005). To minimise this problem, soon after the Kamona strain was isolated, hundreds of vials of it were stored in liquid nitrogen using the method developed by Bedding (1993). *B. siricidicola* could not be stored successfully in liquid nitrogen using methods developed for *Caenorhabditis elegans* or for entomopathogenic nematodes (Popiel *et al*. 1988, Popiel & Vasquez 1991). To store *B. siricidicola* in liquid nitrogen requires suspending aseptic nematodes in sterile 5% glycerol and then evaporating off water in a laminar flow cabinet over several days to achieve nematodes suspended in 50% glycerol before vials, each containing 300 µl of this suspension, are plunged directly into liquid nitrogen. *B. siricidicola* treated in this way have so far survived for 15 years with over 75% viability. Each year, a single vial of original stock is rapidly thawed under running warm water and the resulting suspension added aseptically to young cultures of the symbiotic fungus, *A. areolatum*, on 1/4 strength potato dextrose agar; sub-cultures are from young parent cultures kept in paper bags (to reduce CO_2 accumulation) and mass cultures are made before any decline in infectivity. As soon as the first mass cultures are mature, some are harvested and the resulting nematodes stored in vials in liquid nitrogen. This allows for possible (though unlikely) deterioration of the original stocks and ensures that for the foreseeable future there are stocks of the material originally stored, or at least of material having had only a few generations of further

sub-culturing. Material stored in liquid nitrogen is maintained in three separate locations.

12.6.1.4 Testing for Infectivity

Laboratory cultures or isolates obtained from the field can be tested for their ability to form infective females (which in turn reflects levels of parasitism of sirex that will be obtained from logs inoculated with these isolates). Nematode eggs, harvested under sterile conditions from the mycetophagous cultures of *B. siricidicola*, are placed on *A. areolatum* growing on 0.2% lactic acid/PDA plates inside desiccators containing 10% CO_2. After 10–12 days, the resulting adults are washed off and counted as mycetophagous or infective females. With the defective strain there will be hardly any infective females, whereas with the Kamona strain most adult females will be infective forms.

12.6.1.5 Replacing Defective Strain *B. siricidicola* in the Field

The defective strain is particularly pernicious. R. A. Bedding and J. Calder found that at least six back crosses between Kamona and defective strains (pure Kamona crossed back to progeny of the previous cross) were required before the final hybrids were fully infective (Bedding & Iede 2005). Therefore, it has been an on-going priority to re-introduce Kamona repeatedly into the forests of Victoria, South Australia and southern New South Wales until it can be shown by RAPDs and infectivity tests that the Kamona strain dominates.

12.6.1.6 Temperature Effects

It appears that sirex is well under control in Australia although it is now close to the border of Queensland and has not yet reached the state of West Australia. Areas of pine plantation in both these states as well as northern NSW can be particularly hot in the summer so that sirex-infested trees could heat up enough to kill or disrupt any nematodes breeding within. Thus, Akhurst (1975) found that even at 27.5°C, there was an abortion rate of eggs of 95.9% although after culture at this temperature for 3 weeks, this rate dropped to 15%. Whether temperature adaptation was physiological or genetic is unknown. 30°C was lethal to all stages after initial rearing at 24°C. Although temperatures are buffered within the tree, it is likely that temperatures exceeding 30°C could occur within a tree although pockets of lower temperature might allow survival at some points. Low temperatures that occur in some areas of Australia are unlikely to significantly impact parasitism since high levels of parasitism have been found in Tasmanian forests which are amongst the coldest. However, Akhurst (1975) found that there was negligible egg hatch at 5°C and, even at 10°C, eggs took 13 days to hatch compared to 3 days at 25°C. Considerably more work is needed on the effects of temperature on the life cycle of *B. siricidicola* and this has commenced in Queensland (M Ramsden personal communication).

12.6.1.7 Other Problems

The use of trap trees is an integral part of monitoring and initial introduction of nematodes into new areas. Increasingly, bark beetles are infesting trap trees and rendering them unsuitable for sirex attack. A solution to this situation is being addressed by a study on the use of bark beetle repellents (A Carnegie personal communication).

Particularly in Queensland, there are a number of plantations of *Pinus carabea* and *Pinus carabea* x *Pinus elliottii* hybrids and because sirex has not reached Queensland yet, it has not yet been possible to determine whether this tree species has any effect on the nematode parasitism of sirex. Aspects such as tracheid and bordered pit diameter, resin content, fungal growth rates and production of toxic metabolites of different tree species might have some effect on nematode development and migration within infested trees.

12.6.2 South America

Before it was appreciated that the 198 Sopron strain of *B. siricidicola* had become defective, defective nematodes were introduced into Brazil from Australia in 1989. It was not until 1995 that the Kamona strain was liberated there and so there are likely to be residual populations of defective strain in Brazil and possibly in other South American countries. Kamona strain is not frozen in liquid nitrogen but cultures (hopefully of pure Kamona strain) are re-isolated periodically from the field (ET Iede personal communication). There may be a problem with this approach because, firstly, such isolates could be contaminated with the defective strain and, secondly, the number of generations that the "wild nematodes" will have been cultured in the laboratory will tend to increase each year, while only one parasitic cycle will have occurred. Nematodes isolated from infected sirex in Brazil in 1995 were sent to Argentina, and later cultures, presumably of the Kamona strain, were sent to Uruguay and Chile (Hurley *et al*. 2007).

12.6.3 South Africa

The situation in South Africa is particularly interesting. After sirex was discovered, in stands of *P. radiata* in the Cape Peninsula in 1994, Kamona strain nematodes were introduced and parasitism reached over 96% within 3 years (Tribe & Cillié 2004). However, by 2002 sirex had spread north to the *Pinus patula* plantations of KwaZulu-Natal and by 2007 had killed over 1.5 million trees in 30,000 hectares (Dyer 2007). When sirex-infested trees were inoculated in this region in 2004 and 2005, less than 10% parasitism was recorded from sirex emerging from those trees (Hurley *et al*. 2007) and trees inoculated on Sappi landholdings yielded only 2.1% parasitism in 2004, 9.3% in 2005 and 8.5% in 2006 (Verleur 2007).

Exactly why there should be this huge discrepancy between the Cape (and everywhere else in the Southern Hemisphere) and KwaZulu-Natal has not yet been fully elucidated although there are a number of probable contributing factors. These include rapid drying of infested trees, *P. patula* as the main tree species, incorrect inoculation procedures, poor quality nematodes, nematodes not adapted to the KwaZulu-Natal strain of *A. areolatum*, death of parasitised sirex prior to emergence, interactions between fungus and *P. patula* unfavourable to the nematodes, and, poor initial spread of fungus in infested trees so that nematodes are isolated from the sirex larvae prior to trees drying.

Unlike the Cape, which has winter rainfall, KwaZulu-Natal has summer rainfall and dry warm winters (frequently over 20°C) and this results in sirex-infested trees drying rapidly. Bedding & Akhurst (1974) demonstrated that *B. siricidicola* could not migrate and therefore infect sirex in wood with a water content of less than 30% (= 50% Australian forestry formula). This would certainly be one factor leading to inoculated trees having low parasitism, particularly if trees were inoculated at the same time as in Australia. (Sirex in KwaZulu-Natal emerges at least 2 months earlier than in Australia and therefore infested trees should be inoculated 2 months earlier). When nematodes are introduced by parasitised female sirex, this would occur when the trees had not yet begun to dry and so resulting parasitism should be higher. This is the case, since Verleur (2007) found that female sirex were 54% parasitised in the basal third (the moistest part) of naturally struck trees. It is of course quite possible that parasitism in naturally struck trees could build up much higher as there is increasing parasitism in the wild population (as it does in other regions). In that case, the low levels of parasitism resulting from inoculations could be no more than an expensive nuisance. Whether drying of timber is the only factor producing low levels of parasitism is doubtful since even where moisture levels are much higher than the minimum, parasitism is nowhere near what is to be expected elsewhere.

It appears unlikely that *P. patula* as the tree species could be an important factor since Zondag (1966) reported the first finding of *B. siricidicola*, as heavily parasitising sirex in this tree species: "A nematode disease of *S. noctilio* which caused 95% infection of the adults was discovered in January 1962 in a *Pinus patula* stand in Rotoehu, S. F." Here, a total of 288 males were dissected and 277 were found parasitised (96.2%), while of 81 females, 75 were parasitised (92.6%). Even if tree species is involved in low parasitism then it might be because of some unknown reaction with the South African strain of *A. areolatum*.

That low parasitism from inoculated trees could be operator error or poor quality nematodes was demonstrated to be very unlikely when the operators concerned achieved high levels of parasitism after inoculating sirex-infested *P. radiata* in the Cape Peninsula with nematodes sent from Australia by the same supplier (M Verleur personal communication).

As described below for the USA, strains of *A. areolatum* can vary considerably in their growth rates and suitability as food for *B. siricidicola*. There seems to be no problem with nematodes on this symbiont in the Cape and DNA analysis indicates that *A. areolatum* from KwaZulu-Natal is the same as that from the Cape. However, that does not definitely preclude there being a difference in suitability as food for

B. siricidicola (B Slippers personal communication). Should the fungal strain prove to be a problem, repeated rearing of the Kamona strain on it should lead to better reproduction of the nematode as has been observed for various strains of *B. siricidicola* on various fungal isolates by the author (RA Bedding unpublished data).

12.6.4 North America

The Kamona strain of *B. siricidicola* has been imported into the USA (Williams *et al*. 2007) and results from recent inoculation of logs are pending (D Williams personal communication). In the meantime, and not connected to these inoculations, nematodes have been found parasitizing sirex both in New York state and in southern Canada; these nematodes most likely entered North America with sirex, as occurred in New Zealand. Yu Qing of the Canadian Forest Service has found 30% of several hundred sirex to be parasitised by a *Beddingia* species (Q Yu personal communication), probably *B. siricidicola*, which can be distinguished from the Kamona strain using ITS sequencing (I Leal personal communication). A problem is that the nematodes did not appear to have entered the sirex's eggs (Q Yu personal communication). This means that this particular nematode would have little effect in controlling sirex but it could also have much more serious implications. While failure to enter eggs can be dependent on the strain of *B. siricidicola* concerned, as found in one isolate from New Zealand (Zondag 1975), it can also be a result of the strain of sirex, as found in Belgium (Bedding 1972). In the former case it is possible, although perhaps a little difficult, to replace the nematode with Kamona strain. If the latter, it would make the control of sirex with nematodes unlikely even after extensive searching for new strains. Whether juvenile nematodes enter the eggs of their host is dependent on when the nematodes are released by the parent nematodes into the sirex haemocoel in relation to when the sirex hardens its egg shells (see earlier). The Kamona strain is so "potent" that few eggs are produced by Australian sirex, these are much smaller than normal and juvenile nematodes fill the eggs well before shell hardening; the chances of finding an even more "potent" nematode are slim. However, if required it would be well worth testing the easily obtainable *B. siricidicola* strain from New Zealand, which gave high levels of parasitism in the inoculation trials mentioned above and has a similar effect on sirex's reproductive system.

The symbiotic fungus found in North American sirex is certainly *A. areolatum* but is a different strain from that on which the Kamona nematode is grown in Australia; it not only grows much more slowly than the Australian fungus, but *B. siricidicola* reproduces much more slowly on it (D Williams personal communication). This suggests that the Kamona strain would have some difficulty establishing in sirex-infested trees in North America. Currently the Kamona strain is being passed through many generations on USA fungus (D Williams personal communication) and this should ameliorate the situation.

Even more so than in Australia, using trap trees to introduce nematodes into plantations will be difficult because of a wide variety of bark beetles and other insects that will attack the trees before sirex does; it may therefore be necessary

to inoculate naturally-infested trees *in situ* or distribute inoculated, sirex-infested logs, from areas where sirex is common to where it is not.

Both Canada and parts of USA are subject to extremely low temperatures during winter months and it is not yet known what effect this will have on levels of parasitism or even on nematode survival (Williams *et al*. 2007). Obviously there will be no nematode life cycles for a significant part of the year so that total nematode multiplication and migration within the tree will be at least curtailed; to what extent this may affect final levels of parasitism remains to be seen.

12.7 Discussion

Controlling *Sirex noctilio* with nematodes has become much more complicated with the recent arrival of this insect into Canada and the USA, significant problems with nematode biological control in the KwaZulu-Natal region of South Africa and the possibility of sirex eventually establishing in a further 18 countries (Carnegie *et al*. 2006). Without proper nematode control sirex populations are likely to flourish and that can result in billions of dollars of damage to commercial pine plantations around the world.

Although *B. siricidicola* is a highly successful biological control agent it requires continual management and monitoring to be successful. This is particularly so as sirex is still spreading geographically in almost every country into which it has been introduced as well as into new plantations as they reach an age of 10–12 years. In Australia and South America there is also the problem of replacing any defective strain released earlier and in Canada in replacing the strain that entered the country with sirex. Because of this it is important for each country to have a national sirex coordination committee comprised of forest managers and appropriate scientists and a national sirex strategy that is continually updated as necessary.

Australia's national strategy (Haugen *et al*. 1990) and worksheets (National Sirex Co-ordination Committee 2002) are a good beginning but different climatic conditions, tree species and forestry practices will doubtless require various modifications to be made, which may even vary for different regions within a country. Thus, time of trap tree establishment and nematode inoculation should obviously be much earlier in Brazil and KwaZulu-Natal where sirex emergence is at least 2 months earlier than in Australia and the South African Cape peninsula. The spacing of inoculations and the number of nematodes introduced in each inoculation was carefully worked out to achieve nearly 100% parasitism, but also to result in large parasitised sirex, for sirex-infested *P. radiata* in Tasmania (Bedding & Akhurst 1974). However, for different tree species, where trees dry more rapidly or where long cold winters reduce the possible number of free-living generations, more nematodes in more closely-spaced inoculations may be necessary to achieve the same result and this will need to be determined by experimentation. In areas such as KwaZulu-Natal where infested *P. patula* dry out very rapidly, it may even be necessary to inoculate sirex-infested material from areas where this is plentiful, as early as possible, and

then collect the inoculated logs at central points for periodical water spraying prior to distributing them where required.

It would be tempting to consider the introduction of new or multiple strains of *B. siricidicola*, perhaps better adapted to certain forest conditions in a particular area. This should be avoided if at all possible. Firstly, the Sopron, and later the Kamona, strains were carefully selected over many years from hundreds of isolates from all over the world at a huge cost (these strains are thought to be equivalent but were isolated from the field at different times and sites). Secondly, most natural strains are likely to be adapted so that they do as little harm to their host's population as possible, which is not what is required. Finally, once an inferior strain has been released it can be very difficult if not impossible to replace it. Nevertheless, if the Sopron and Kamona strains do not enter the eggs or do not significantly reduce egg numbers of a particular strain of sirex (that has been introduced to a region/country or has arisen from mutation of normal sirex) then there is no alternative but to look for another nematode strain that does. The development of such a strain of sirex from "normal" sirex is of course a possibility and that would become a major problem. However, it is more likely that an ineffective nematode strain would develop since nematodes have many generations each year compared with usually only one for sirex.

There is a still a considerable body of applied research required to make nematode control fully effective in the various regions where sirex is or is likely to become a problem. These include the effect of temperature, tree species, fungal interaction, effect of other insects, inoculation strategies for different situations, trap tree protection and molecular probes for sirex and nematode strains.

Presumably *S. noctilio* will remain a major pest of pine trees throughout the world for the foreseeable future but nematodes should be an effective means of controlling it for hundreds of years to come, provided the situation is continually monitored and managed appropriately.

References

Akhurst RJ (1975) A study of the free-living phase of *Deladenus*, nematodes parasitic in woodwasps. MSc Thesis, University of Tasmania

Bedding RA (1967) Parasitic and free-living cycles in entomogenous nematodes of the genus *Deladenus*. Nature London 214:174–175

Bedding RA (1968) *Deladenus wilsoni* n.sp. and *D. siricidicola* n.sp. (Neotylenchidae), entomophagous nematodes parasitic in siricid woodwasps. Nematologica 14:515–525

Bedding RA (1972) Biology of *Deladenus siricidicola* (Neotylenchidae), an entomophagous nematode parasitic in siricid woodwasps. Nematologica 18:482–493

Bedding RA (1975) Five new species of *Deladenus* (Neotylenchidae), entomophagous-mycetophagous nematodes parasitic on siricid woodwasps. Nematologica 20:204–225

Bedding RA (1979) Manipulating the entomophagous-mycetophagous nematode, *Deladenus siricidicola* for the biological control of the woodwasp *Sirex noctilio* in Australia. In: Waters WE (ed) Current topics in forest entomology. USDA Forest Service Gen Tech Pap WO-8. pp 144–147

Bedding RA (1984) Nematode parasites of Hymenoptera. In: Nickle WR (ed) Plant and insect parasitic nematodes. Marcel Dekker, New York. pp 755–795

Bedding RA (1992) Strategy to overcome the crisis in control of sirex by nematodes. Austral For Grower Summer 1991/92:15–16

Bedding RA (1993) Biological control of Sirex noctilio using the nematode *Deladenus siricidicola*. In: Bedding RA, Akhurst RJ, Kaya HK (eds) Nematodes and the biological control of insect pests. CSIRO Publ, East Melbourne, Australia. pp 11–20

Bedding RA, Akhurst RJ (1974) Use of the nematode *Deladenus siricidicola* in the biological control of *Sirex noctilio* in Australia. J Aust Entomol Soc 13:129–135

Bedding RA, Akhurst RJ (1978) Geographical distribution and host preferences of *Deladenus* species (Nematoda: Neotylenchidae) parasitic in siricid woodwasps and associated hymenopterous parasitoids. Nematologica 24:286–294

Bedding RA, Iede ET (2005) Application of *Beddingia* for Sirex wood wasp control. In: Grewal PS, Ehlers R-U, Shapiro-Ilan DI (eds) Nematodes as biological control agents. CABI Publ, Cambridge, MA. pp 385–399

Blinova SL, Korenchenko EA (1986) *Phaenopsitylenchus lacicis* g.n. and sp.n. (Nematoda: Phaenopsitylenchidae fam. N.) parasite of *Phenops guttulata* and remarks on taxonomy of nematodes of the superfamily Sphaerularioidea. Acad Nauk SSSR Trudy Gelmint Lab 34:14–23

Calder J, Bedding RA (2002) Standard operating procedures for production, experimental analysis and quality assurance testing of *Beddingia siricidicola*. National Sirex Co-ordination Comm, Canberra, Australia

CanFI2001 Database (2007) Canadian Forest Service, Natural Resources Canada, http://cfs.nrcan.gc.ca/subsite/canfi/index-canfi [accessed March 2008]

Carnegie AJ, Waterson DG, Eldridge RH (2005) The history and management of sirex wood wasp, *Sirex noctilio* (Hymenoptera: Siricidae), in pine plantations in New South Wales, Australia. NZ J For Sci 35:3–24

Carnegie A J, Matsuki M, Haugen DA, Hurley BP, Ahumada R, Klasmer P, Sun J, Iede ET (2006) Predicting the potential distribution of *Sirex noctilio* (Hymenoptera: Siricidae), a significant exotic pest of *Pinus* plantations. Ann For Sci 63:119–128

Coutts MP (1969a) The mechanism of pathogenicity of *Sirex noctilio* on *Pinus radiata*. 1, Effects of the symbiotic fungus *Amylostereum* sp. (Thelophoraceae). Aust J Biol Sci 22:915–924

Coutts MP (1969b) The mechanism of pathogenicity of *Sirex noctilio* on *Pinus radiata*. 2, Effects of *S. noctilio* mucus. Aust J Biol Sci 22:1153–1161

Dyer D (2007) An overview of the national *Sirex* control strategy in South Africa. Internatl Sirex Symposium, Pretoria, South Africa 9–16 May 2007. p 36

Gaut IPC (1969) Identity of the fungal symbiont of *Sirex noctilio*. Aust J Biol Sci 22:905–914

de Groot P (2007) An overview of the *Sirex noctilio* situation in Canada. Internatl Sirex Symposium, Pretoria, South Africa 9–16 May 2007. p 30

Haugen DA, Underdown MG (1990) *Sirex noctilio* control program in response to the 1987 Green Triangle outbreak. Aust Forest 53:33–40

Haugen DA, Bedding RA, Underdown MG, Neumann FG (1990) National strategy for control of *Sirex noctilio* in Australia. Austral For Grower 13 No 2

Hoebeke ER, Haugen DA, Haack R (2005) *Sirex noctilio*: Discovery of a Palearctic siricid woodwasp in New York. Newsl Mich Entomol Soc 50:24–25

Hurley BP, Slippers B, Hatting HJ, Croft PK, Wingfield MJ (2007) The *Sirex* control program in the eastern parts of South Africa: lessons from research efforts between 2004–2006. Internatl Sirex Symposium, Pretoria, South Africa 9–16 May 2007. p 38

Iede ET, Penteado S do RC, Schaitza EG (1998) *Sirex noctilio* in Brazil: detection, evaluation, and control. In: Proc Conference Training in the control of *Sirex noctilio* by the use of natural enemies, Colombo, Brazil. pp 45–52

Iede ET, Klasmer P, Penteado S do R.C (2000) *Sirex noctilio* in South America: distribution, monitoring and control. In: XXI Internatl Congr Entomol. Foz do Iguacu, Aug. 2000. Embrapa Soja Anais, Londrina-PR. p 474

Iede ET, Penteado S do RC, Filho WR (2007) The woodwasp *Sirex noctilio* in Brazil – Monitoring and control. Internatl Sirex Symposium, Pretoria, South Africa 9–16 May 2007. p 20

Madden JL (1968) Physiological aspects of host tree favourability for the woodwasp, *Sirex noctilio* F. Proc Ecol Soc Aust 3:147–149

Madden JL (1974) Oviposition behaviour of the woodwasp, *Sirex noctilio* F. Aust J Zool 22:341–351

Madden JL (1977) Physiological reactions of *Pinus radiata* to attack by the woodwasp, *Sirex noctilio* F. (Hymenoptera: Siricidae). Bull Ent Res 67:405–426

Madden JL, Irvine CJ (1971) The use of lure trees for the detection of *Sirex noctilio* in the field. Aust Forest 35:164–166

National Sirex Co-ordination Committee (2002) National Sirex Control Strategy Operations Worksheets

Poinar Jr GO, Jackson TA, Bell NL, Wahid MB (2002) *Elaeolenchus arthenonema* n. g., n. sp. (Nematoda: Sphaerularioidea: Anandranematidae n. fam.) parasitic in the palm-pollinating weevil ***Elaeidobius kamerunicus*** Faust, with a phylogenetic synopsis of the Sphaerularioidea Lubbock, 1861. System Parasitol 52:219–225

Popiel I, Vasquez EM (1991) Cryopreservation of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*. J Nematol 23:432–437

Popiel I, Holtemann KD, Glazer I, Womersley C (1988) Commercial storage and shipment of entomogenous nematodes. International Patent No. WO 88/011344

Remillet M, Laumond C (1991) Sphaerularioid nematodes of importance in agriculture. In: Nickle WR (ed) Manual of agricultural nematology. Marcel Dekker, New York. pp 967–1024

Riding IL (1970) Microvilli on the outside of a nematode. Nature Lond 226:179–180

Schneeberger NF (2007) Response to the recent find of *Sirex noctilio* in the United States. International Sirex Symposium, Pretoria, South Africa 9–16 May 2007. p 20

Spadberry JP, Kirk AA (1978) Aspects of the ecology of siricid woodwasps (Hymenoptera: Siricidae) in Europe, North Africa and Turkey with special reference to the biological control of *Sirex noctilio* F. in Australia. Bull Ent Res 68:341–359

Sutherst RW, Maywald GF, Yonow T, Stevens PM (1999) CLIMEX: predicting the effects of climate on plants and animals. CSIRO Publ, Melbourne, Australia

Tribe GD, Cillié JJ (2004) The spread of *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) in South African pine plantations and the introduction and establishment of its biological control agents. African Entomol 12:9–17

Verleur M (2007) Validation for mass inoculations with *Beddingia siricidicola*, despite apparent low inoculation generated parasitism rates. International Sirex Symposium, Pretoria, South Africa 9–16 May 2007. p 40

Williams D, Mastro V, Downer K (2007) Establishment of *Beddingia siricidicola* for biological control of *Sirex noctilio* in the United States: Questions, issues, and challenges. International Sirex Symposium, Pretoria, South Africa 9–16 May 2007. p 32

Wood MS, Stephens NC, Allison B, Howell CI (2001) Plantations of Australia – a report from the National Plantation Inventory and the National Farm Forest Inventory (abridged version). National Forest Inventory, Bureau of Rural Sciences, Canberra

Zondag R (1962) A nematode disease of *Sirex noctilio* (F.). Interim Res. Rep., New Zealand Forest Service 1–6

Zondag R (1966) Observations on a nematode disease of *Sirex noctilio* (F.). NZ For Res Inst, For Entomol Report No 19

Zondag R (1975) A non-sterilising strain of *Deladenus siricidicola*. NZ For Res Inst Report 1974. Rotorua, NZ. pp 51–52