NEMATODES FOR THE BIOLOGICAL CONTROL OF THE WOODWASP, *SIREX NOCTILIO*

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ABSTRACT

The tylenchid nematode *Beddingia* (*Deladenus*) *siricidicola* (Bedding) is by far the most important control agent of Sirex noctilio F., a major pest of pine plantations. It sterilizes female sirex, is density dependent, can achieve nearly 100 percent parasitism and, as a result of its complicated biology can be readily manipulated for sirex control. Bedding and Iede (2005) gave a comprehensive account of this nematode and its use in Australia and South America.

Throughout the 1960s and early 1970s, CSIRO 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 any 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 and Akhurst 1978). Only one species of nematode, *B. siricidicola*, was found to be suitable for sirex control and this has now been released and established in Australia, New Zealand, Brazil, Uruguay, Argentina and South Africa.

B. siricidicola is extraordinary in having two separate life cycles associated with two morphologically very different adult female types (Bedding1967, 1968, 1972). In the parasitic cycle, 5 to 20 mm long, cylindrical, often green-colored females release thousands of juvenile nematodes into the body cavity of adult sirex wasps. In the free-living cycle 1 to 2 mm long females feed on the symbiotic fungus, *Amylostereum areolatum* (Fr.) Boidin as it grows in the tree, and lay eggs in the tracheids (wood fibers). (These two types of females are so morphologically different from each other that each on its own would have been placed in a separate family of nematodes. Poinar et al. [2002] have now placed both forms of *Beddingia* species in a new family, the Beddingiidae).

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, this is a dead end but female sirex are effectively sterilized because, as well as ovarial development being suppressed to various degrees, each egg that is produced is filled with up to 200 juvenile nematodes. A 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 unparasitised sirex can eventually become infected with nematodes, but this is only made likely by the many intervening free-living cycles which enable the nematode to breed through the tree in vast numbers. It is only when juvenile 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 preparasitic kind of female rather than the fungal-feeding form. After mating the infective female uses its large anterior spear-like stylet to bore into sirex larvae and within a few weeks of penetrating a larva the nematode grows up to 1000-fold in volume. It now remains, often for several months, relatively unchanged, until the sirex larva pupates. Then the nematode's reproductive system 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.

The fungal-feeding life cycle is not only important in enabling high levels of parasitism in sirex

populations, it can be used to manipulate the nematode for biological control purposes. Initially it was possible to maintain a library of hundreds of isolates of the various nematode species and strains on fungi growing on agar slopes which were kept under refrigeration for many years while being evaluated as control agents. In addition, once the best species/strain had been selected, it could be sterilely mass reared, for liberation, in flasks of autoclaved wheat/rice inoculated with *A. areolatum*.

Selecting the best nematode species for liberation from the library of stored isolates was relatively simple; only two species fed on *A. areolatum* (the symbiont of *S. noctilio*) and one of these *B. wilsoni* was excluded because it parasitised rhysinne parasitoids. However, there still remained hundreds of isolates of *B. siricidicola* from 20 countries, 10 tree species and four sirex species. The best strain, from Sopron in Hungary, was selected because it totally sterilizes female *S. noctilio*, gives the highest levels of parasitism and unlike strains that fulfilled the first two criteria, has little effect on the size of sirex emerging from logs correctly inoculated with it. (The large size of parasitized sirex is important because they fly further, visit more trees, lay more nematode filled eggs and live longer than smaller ones.)

Once nematodes are well established in a sirex population, the parasitized female sirex perform the main task of spreading them. However, initially (where less than 1 percent of trees are naturally killed by sirex) the nematodes have to be introduced into new plantations by inoculating 'trap trees' set up at easily accessible strategic points. A group of 5 to 10 preferably low quality trees near the roadside are selected for each compartment (50 acres or so) a few months before the sirex flight season, and injected with just enough weedicide, so that they die slowly and are particularly attractive to sirex. Once sirex populations are established in these trap trees, the trees are felled and inoculated with nematodes, suspended in polyacrylamide gel, at a rate of 2000 per inoculation hole with holes about 1 cm deep and 30 cm apart.

Important factors are:

- Cutting the holes cleanly with a specially designed hammer/punch so that nematodes can easily enter the tracheids
- Using gel so that the nematodes are suspended within the hole for adequate time
- Introducing the nematodes when the wood is adequately moist
- Spacing of inoculation holes and number of nematodes introduced into each hole which has been carefully tested to provide maximum parasitism (over 98%) and minimum competition between fungal feeding nematodes and the fungal feeding sirex larvae so that the latter grow to full size (Bedding and Akhurst 1973)
- Because parasitized female sirex rarely leave the compartment of liberation it is important to introduce nematodes to each forest compartment and repeat inoculations, if required, each year until over 10 percent parasitism occurs in each compartment

Where sirex killed trees exceed 1 percent of the total, all naturally infested trees in every 5th row (20 percent) should be inoculated to achieve adequate control. Examples of successful treatment of sirex populations that were out of control because nematodes were not previously present are:

A) The Mt. Helen plantation in Tasmania in 1974 where 10 percent of all mature trees had been dying for each of the previous 6 years, all dead trees in every 10th row were inoculated. In 1975 there was near 100 percent parasitism and in 1976 there were negligible sirex.

B) The "Green Triangle" in South Australia/ Victoria in 1987 where there was up to 80 percent tree death in some areas, 166,000 sirex-killed trees (from every 5th row) were inoculated and by 1989 nearly 100 percent parasitism was followed by a population collapse; sirex has been rare in that region ever since (Haugen and Underdown 1990)

C) In a 12,000-ha plantation in Encruzilhado do Sul in Brazil, where sirex infested about 30 percent of trees in some compartments in

1991, nematodes were released from 1990 to 1993, resulting in levels of parasitism of 45 percent in 1991, 75 percent in 1992 and more than 90 percent in 1994. In 1995 it was difficult to find any sirex-infested trees in this area (Bedding and Iede 2005).

There has been one major problem with nematode control of sirex. Because *B. siricidicola* 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 (Bedding and Iede 2005). Unfortunately the situation did not become apparent until this defective strain had been liberated for many years. However, in 1990 the original strain was re-isolated from where it had been liberated in Kamona, Tasmania in 1971 and stocks have been maintained since then in liquid nitrogen for re-establishment of the cultures used for all new introductions. In addition it has been found recently that even Kamona strain tends to deteriorate after several months of sub-culturing so that it is imperative to return to stocks stored in liquid nitrogen at least once at the beginning of each season. Nevertheless, replacing the defective strain in the field has proved very difficult particularly since it requires several back-crosses to the "Kamona" strain to completely revive infectivity. A program of strain replacement is still continuing in Australia aided by the development of RAPD molecular identification enabling separation of the defective and Kamona strains from field collected samples. Infectivity tests are also conducted on field collected samples by exposing acidic potato dextrose agar plates inoculated with *A. areolatum* and nematode eggs to an atmosphere containing 12 percent CO_2. (In this situation fewer than 10 percent of eggs from the defective strain give rise to parasitic females whereas over 80 percent of eggs from the Kamona strain do so.)

Now that *S. noctilio* has been found in both the USA and Canada it will be essential to use *B. siricidicola* to help protect the many millions of acres of highly susceptible pine there. Only top quality, Kamona strain nematodes should be used and their quality should be maintained using liquid nitrogen storage from the outset.

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