Manipulating the Entomophagous-Mycetophagous Nematode, *Deladenus Siricidicola*, for Biological Control of the Woodwasp *Sirex Noctilio* in Australia

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Introduction

During the nineteenth century, a scarcity of endemic softwoods in Australia led to the introduction of various exotic pines. One of these, *Pinus radiata* D. Don (introduced in 1857), has flourished under Australian conditions and forms the bulk of commercial plantations which now exceed half a million hectares with an annual return approaching $250 million; and the industry continues to expand rapidly. However, in 1952, *Sirex noctilio* F. was discovered in Tasmania, having been introduced probably via shipment of timber from New Zealand, and, within a decade, it has killed some 40 percent of radiata pine trees in a forest near Hobart. Thus, what is probably the most susceptible tree species (from California) was exposed to the most virulent siricid (originally from Europe) in the absence of any of its natural biological control agents.

When it was found in 1961 that *S. noctilio* had reached the mainland of Australia, the National Sirex Fund was established and one of the most extensive worldwide searches for natural enemies instituted. When initial attempts at eradication proved unsuccessful, a massive programme of "Search and Destroy" was carried out by the Forests Commission, Victoria, while a comprehensive research programme was instituted on various other aspects of control, including attractants, selective breeding of resistant trees, and the introduction of insect and nematode parasites. Despite all efforts to contain them, infestations of *S. noctilio* have spread steadily from the initial outbreak area near Melbourne over many thousands of hectares and are now only about 60 km from the vast coniferous forests surrounding Mt. Gambier (80,000 ha).

Following the discovery of nematodes infecting *S. noctilio* in New Zealand (Zondag 1962), CSIRO commenced investigations in 1965 into nematode parasites of various siricids and their parasitoids. The life history of these nematodes was shown by Bedding (1967, 1972a, and 1976b) to be an extraordinary one, involving profound female dimorphism associated with free-living mycetophagous and parasitic life cycles; seven species of *Deladenus* (Neotylenchidae) parasitic on siricids were described by Bedding (1968, 1974), and a detailed account of the use of *Deladenus siricidicola* Bedding in the biological control of *S. noctilio* was given by Bedding and Akhurst (1974).

Biology of S. noctilio

When female *S. noctilio* (usually several on one tree) drill into the wood of living pine trees to oviposit, they also insert toxic mucus and spores of the symbiotic fungus *Amylostereum areolatum* (Fr.) Boidin. The mucus and fungus kill susceptible trees (Coutts 1969) and *S. noctilio* larvae bore through and feed up on the fungus infected wood. Adult *S. noctilio*, which emerge 1 to 3 years after oviposition, are subject to predation by birds while immature stages are attacked by various ichneumonid, cynipid, and nematode parasites.

Biology of D. siricidicola

Shortly after investigations by CSIRO into the biology of *D. siricidicola* commenced, it was found that when sections of logs, from which all siricids had emerged 2 years previously, were placed in Baermann funnels, these yielded large numbers of nematode juveniles apparently identical to those from siricid hosts. This suggested that the nematodes were possibly feeding within the

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wood on siricid symbiotic fungus. Accordingly, juvenile nematodes removed aseptically from siricid hosts were placed onto cultures of the fungus *Amylostereum areolatum* growing on potato dextrose agar plates. The nematodes fed, matured, and laid eggs on the growing front of the fungus, and it was found that monoxenic cultures could be subcultured indefinitely without intervention of the siricid host; this, despite the fact that the original nematodes were fully parasitic in the host's haemocoel.

Parasitized *S. noctilio* larvae contain 1 to 100 cylindrical adult female nematodes, often bright green, 5 to 25 mm in length. The nematode's reproductive system remains undeveloped (about 0.5 mm in length) until the onset of host pupation and then expands rapidly, producing several thousand eggs which hatch within the parent; juvenile nematodes escape into the haemocoel of the host pupa and migrate into its reproductive organs entering the ovaries and eggs of female *S. noctilio* and testes of the male. Female *S. noctilio* are sterilized but since spermatozoa have already passed into the vesiculae seminales before invasion of the testes by juvenile nematodes, fertility in the male is unimpaired.

Parasitized female *S. noctilio* oviposit readily, implanting symbiotic fungus and eggs each containing up to 200 juvenile nematodes. These migrate from the hosts' eggs, feed on the developing fungus and grow into adult free-living nematodes (1.3 to 2.5 mm in length) which lay many eggs within the tracheids around *S. noctilio* oviposition holes. After the tree dies and as the wood dries out, the symbiotic fungus spreads throughout it and nematodes breed within tracheids, resin canals, and even beneath the bark. In these relatively monoxenic areas where fungus is sparse, juveniles develop only into mycetophagous forms, but in the vicinity of *S. noctilio* larvae (progeny of uninfected females attacking the same tree), where bacteria abound, juvenile nematodes may develop into adult infective females which are quite unlike the mycetophagous females. After insemination (by males morphologically identical to those fertilizing mycetophagous females, but having microspermatozoa), infectives penetrate *S. noctilio* larvae and grow up to a thousandfold in volume within a few weeks.

Because the relatively few nematodes introduced by parasitized *S. noctilio* can breed up through several generations so that they eventually occur in large numbers throughout the tree, they are able to achieve levels of parasitism often approaching 100 percent.

Utilizing D. siricidicola to Control S. noctilio

A thorough knowledge of the biology of this nematode has proven essential for its successful manipulation to control *S. noctilio* on a large scale. Aspects of greatest importance are its ability to sterilize female *S. noctilio*, the free-living cycle which can be utilized for establishing and maintaining cultures and mass rearing; its specificity to the symbiotic fungus (which restricts it to the environment of siricid larvae); and an inability to affect important insect parasitoids.

The importance of being able to culture readily and therefore store nematodes after removal from their hosts cannot be over-rated. Some 22,000 insects of 34 different species from 31 tree species and hundreds of localities in 28 countries have been dissected and nematodes cultured (Bedding and Akhurst, 1978). This has meant that an armory of many different species and strains of *Deladenus* could be stored for evaluation over several years. It has made possible the rapid screening of hundreds of cultures for new species by crossbreeding experiments on fungal culture (Akhurst 1975), and has enabled extensive testing to determine the effect of various nematode species and strains on insect parasitoids.

Evaluation

Important aims of evaluation have been to determine which species and strains will parasitize and sterilize *S. noctilio* without harming the insect parasitoids and which will be the most efficient parasites.

Of the seven species of nematodes isolated from siricids, it was soon found that only two could successfully utilize the fungus *A. areolatum* (as opposed to *A. chailletii* from most siricid species) and of these only *D. siricidicola* gave good parasitism of *S. noctilio* without harming the parasitoids. However, many strains of *D. siricidicola* were cultured from Europe, Japan, and New Zealand. After eliminating those few that parasitized but did not sterilize female *S. noctilio*, several strains were tested in comprehensive experiments to determine the relative levels of parasitism achieved. In these experiments, some strains were obviously inferior to others and were eliminated.

Since the nematode relies for its distribution on *S. noctilio* females, the extent to which parasitism affects flying and oviposition capabilities is important. Comprehensive experiments using flight mills, although they showed that flight capabilities were highly variable, indicated that there was no significant difference between parasitized and unparasitized *S. noctilio* of the same size (Bedding and Akhurst, in preparation). However, the largest females of *S. noctilio* flew up to 10 times as far as the smallest, and there is a relationship between decrease in size of *S. noctilio* and the presence of nematodes. Various strains of *D. siricidicola* differed in this respect and two strains which had little significant effect on the size of emerging *S. noctilio* as well as giving the highest levels of parasitism were therefore selected for liberation. Size is, of course, also important in relation to the number of eggs laid and the number of juvenile nematodes produced within the host.

Mass Rearing Nematodes

The use of host insects, which have a life cycle of from 1 to 3 years, to rear large numbers of nematodes for release would be both tedious and expensive; taking advantage of the nematodes free-living cycle is far more efficient. Although potato dextrose agar culture plates will each produce only a few thousand nematodes, these may be used to inoculate flasks containing autoclaved wheat (100g wheat and 150 ml water per 500 ml flask), which yield from 3 to 10 million juvenile nematodes within 4 to 6 weeks after incubation at 24°C (Bedding and Akhurst 1974). Since the contents of a single flask are sufficient to inoculate about 100 metres of *S. noctilio*-infected tree trunk, no attempts were made to scale up the process.

Inoculation of Trees

In order to introduce *D. siricidicola* to *S. noctilio* infestations, a number of easily accessible infested trees scattered throughout the infested area are usually felled and inoculated as they lie. Most female *S. noctilio* emerging from correctly inoculated trees are parasitized, and these oviposit on trees which usually are also attacked by wild unparasitized *S. noctilio*. The techniques used for inoculation are important; early methods resulted in low, variable, or no parasitism, but the methods currently used regularly produce over 99 percent parasitism.

Both preparation of inoculation holes and the medium used to inoculate are of critical importance: normal drilling of wood results in twisted tracheids which impede nematode entry; water suspensions are rapidly adsorbed leaving the nematodes to desiccate. Although chisel cuts proved fairly suitable for nematode entry a wad punch mounted to form a hammer has proven the most efficient tool for making inoculation holes and aerated 12 percent gelatin with 4000 nematodes per ml the most satisfactory medium (Bedding and Akhurst 1974). (Other gel media, such as agar, were much less satisfactory because nematodes failed to emigrate). A spacing of one inoculation per metre was found adequate to produce almost 100 percent parasitism. Heavier inoculation resulted in early competition between nematodes and *S. noctilio* larvae for fungal food and thus smaller *S. noctilio* females in the next generation.

Field Liberations

Experimental liberations in Tasmania (Bedding and Akhurst, in preparation) have indicated that *D. siricidicola* establishes, spreads, and achieves high levels of parasitism rapidly. In a 400 ha forest in Northern Tasmania with a low infestation of *S. noctilio*, some 50 parasitized females were allowed to emerge from a single point in one corner of the forest during 1970. By 1972, nematodes had spread to 37 percent of siricid infested trees in the whole forest and 92 percent in the compartment of liberation (over 70 percent of *S. noctilio* emerging from any nematode infested tree were parasitized). Two years later, over 70 percent of all trees contained nematodes (with over 90 percent of *S. noctilio* emerging from these parasitized), and the number of trees killed by *S. noctilio* in the next year dropped dramatically. As of this writing, no trees have been found that were killed in 1976. In a nearby forest of similar size, several thousand trees were killed annually by *S. noctilio*, but after a heavy inoculation program designed to achieve 10 percent parasitism during the first year (1972), over 90 percent parasitism resulted in the following year. The year afer this, only 200 trees were killed by *S. noctilio*; the next year only five, and now *no* fresh *S. noctilio*-killed trees can be found either by ground or aerial survey. The nematode has also spread naturally to other nearby forests 2, 7, 8, and 13 kilometres away, and the level of parasitism is already high.

During 1970, some 1000 inoculated billets were sent from Tasmania to the Forest Commission, Victoria for distribution in *S. noctilio*-infested areas. Since then, hundreds of millions of nematodes in oxygenated water have been sent to Victoria and, in a major program of liberation by the Forests Commission (which at one stage had 10 mobile crews searching for and inoculating trees in infested forests, nematodes have been liberated throughout most of the *S. noctilio*-infested areas in Victoria. *D. siricidicola* is now established over most of Victoria, and high levels of parasitism are already in evidence.

The program of nematode control is complemented by and coordinated with a similar one for insect parasitoids (Taylor 1976), and it is expected that the manipulation of both nematodes and parasitoids will become standard forestry practice. In this respect, nematodes are most easily and cheaply reared in the laboratory, but both kinds of agents can usually also be introduced to new *S. noctilio* infestations by transfer of infected billets from one forest to another. Comprehensive evaluation experiments are being continued in both Tasmania and Victoria, but it will be many years before the full impact of biological agents can be assessed.

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