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Reduced parasitism of Sirex noctilio *in radiata pines inoculated with the nematode* Beddingia siricidicola *during 1974-1989*

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Summary

Artificial inoculations of radiata pines with the nematode *Beddingia siricidicola* from 1985 to 1988 have resulted in less than 30% of the emerging *Sirex noctilio* being parasitised by these nematodes. In contrast, the original inoculation procedure, developed in 1974, consistently produced greater than 95% parasitism. Because various components of the inoculation procedure have been changed since 1974, a study was started during 1989 to investigate the effects of gelatin concentration of the nematode carrier, of hammer type used to make inoculation holes, and of moisture content of the wood on parasitism rates by the nematode. Results indicated that these factors were not responsible for the low rates of parasitism. The infective capacity of nematodes produced in laboratory cultures needs to be investigated.

Introduction

Sirex noctilio F. (Hymenoptera: Siricidae) is a major pest of radiata pine (*Pinus radiata* D. Don) plantations in Australia. Substantial economic damage has been caused by sirex in Tasmania, Victoria, South Australia, and New South Wales (Madden 1975, McKimm and Walls 1980, Haugen 1990). The risk of sirex increasing its geographic distribution in Australia is high. There are more than 250,000 ha of uninfested pine plantations, mainly in northeastern New South Wales, southeastern Queensland, and southwestern Western Australia, that are susceptible to colonization by sirex. A comprehensive strategy was prepared to aid forest managers in implementing an effective control program for sirex (Haugen *et al.* 1990). A primary component of this strategy is the release and establishment of biological control agents.

A parasitic nematode, *Beddingia (= Deladenus) siricidicola* (Bedding) (Neotylenchidae), is the key biological control agent of sirex in Australia (Bedding and Akhurst 1974, Taylor 1981, Neumann and Morey 1984). Bedding and Akhurst (1974) developed a procedure for liberating this nematode into field populations of sirex. Sirex-attacked trees were felled, holes were punched with the Gould hammer every 30 cm along the bole of a tree, and these holes were filled with a 12% gelatin foam that contained nematodes (ca. 2000 nematodes/hole). This inoculation procedure consistently resulted in parasitism of over 95% of the emerging sirex.

From 1985 to 1989, evaluations of caged logs from inoculated trees in southeastern South Australia and southwestern Victoria have shown relatively low percentages of sirex parasitised by nematodes (Table 1). The original inoculation procedure had been modified since its development (Table 2). These changes appear to have been made without testing for effects on rates of parasitism. Bedding (1979) reported that early methods resulted in variable and low parasitism, and he emphasized the importance of proper inoculation techniques.

In this paper, results are presented on the effects of gelatin concentration, type of hammer, and moisture content of the wood on the rates of parasitism by the nematode.

Materials and methods

The study site was a 14-year-old radiata pine plantation at Noolook Forest Reserve, near Robe, South Australia. Nematodes were first introduced into this geographically isolated plantation starting in 1987. Evaluations of caged logs from uninoculated

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trees in this plantation during the 1988/89 emergence season showed that less than 5% of the sirex were parasitised by nematodes.

During July 1989, 55 sirex-infested trees were selected from a compartment in this plantation. Two logs, each 3 m long, were cut from the mid-section of each tree. Ends of the logs were coated with a timber sealer to retard moisture loss. Three cross-sectional disks, 2 cm in thickness, were cut from each tree to assess the moisture content at the time of inoculation (Fig. 1). The moisture content of each disk was assessed as a percentage of oven-dry weight (ODW), and a mean was calculated for each log.

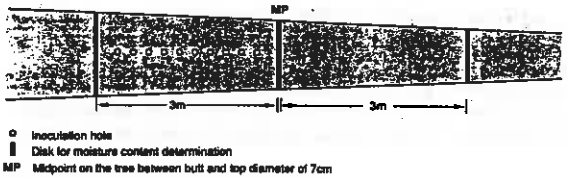

Figure 1. Schematic diagram of a sample tree (top view) indicating an inoculated log, an uninoculated log, and disks for moisture content determination.

One of the five inoculation treatments was randomly assigned to one of the logs from each tree, and the other log was kept as an uninoculated control. The five inoculation treatments were gelatin concentrations of 3%, 6%, 9%, and 12% applied to holes made by a SWAT hammer (Haugen and Underdown 1990) and a gelatin concentration of 12% applied to holes made by the Gould hammer (Bedding and Akhurst 1974). Holes were punched at 30 cm intervals along the logs and filled with a gelatin/nematode mixture (ca. 2000 nematodes per hole) on 26 July 1989. Nematodes were obtained from the laboratory cultures at the Keith Turnbull Research Institute (Department of Conservation and Environment, Victoria).

During December 1989, the 110 logs were collected from the compartment and caged individually in 200 L drums (tops covered with insect-proof netting). Cages were checked weekly until May 1990 for emerged sirex. All sirex that emerged were dissected to determine the percentage parasitised by the nematode.

Results

Emergences from the 110 cages ranged from 0-144 sirex (mean = 35.2/cage). Nine of the cages with

Table 1. Percentage of sirex parasitised by *Beddingia siricidicola* from inoculated and uninoculated trees in southeastern South Australia and southwestern Victoria[1].

		Percentage of sirex parasitised				
		Inoculated		Uninoculated		Net gain of inoculation
Year[2]	No. of units[3]	mean	range	mean	range	
1985/86	5	63	38–85	—	—	—
1986/87	2	17	16–18	0	0	17
1987/88	51	26	0–93	15	0–90	11
1988/89	11	47	12–71	34	2–87	13

[1] Sources of data:
1985/86 — Australian Forestry Council 1986
1986/87 — Woods & Forests, unpublished report, August 1987
1987/88 — Haugen, unpublished data
1988/89 — Haugen, unpublished data.

[2] Year of evaluation (summer/autumn).

[3] Number of evaluation units (i.e. locations, compartments, or cages).

Table 2. Variations in procedures[1] used to inoculate the nematode *Beddingia siricidicola* into sirex-infested trees.

References	Gelatin concentration (%)	Hammer type	No. nematodes per hole	Hole spacing cm	Infection of sirex %
Bedding & Akhurst 1974	12	Gould	2000	30	>95
Neumann & Minko 1981	NG	Gould	2500	20–25	NG
Neumann *et al.* 1987	5	Gould	1800	30–40	NG
Anonymous 1988	NG	NG	600–1200	25–33	NG
Haugen & Underdown 1990	6	SWAT	1000	20	<25

[1] NG = information not given.

uninoculated logs and 17 cages with inoculated logs had less than 10 sirex dissected. Paired cages were deleted from the data set if either of the cages had less than 10 sirex dissected, which left 34 pairs of cages for the analyses.

Parasitism by the nematode ranged from 0-100% (mean = 46%) from the uninoculated logs. Only 8 of the 34 uninoculated logs had no sirex parasitised by nematodes. Twenty-one of the logs had greater than 25% of the sirex parasitised. Parasitism rates were summarized for the various gelatin concentrations and hammer types (Table 3). Parasitism rates among the treatments for inoculated logs were not significantly different (ANOVA, $p > 0.05$). However, the high levels of parasitism in the uninoculated logs confounded the analyses for treatment effects in the inoculated logs and possibly obscured any true differences among treatments.

The mean moisture content of the logs was 44.7% (SD = 8.8%) with a range from 33% to 72%. Bedding and Akhurst (1974) recommended that logs should have moisture contents above 50% ODW when inoculated. In this study, logs with moisture contents above 50% and inoculated with nematodes in 12% gelatin (using either hammer) had less than 30% of the emerging sirex parasitised. However, some logs with moisture contents between 35-50% had greater than 75% of the sirex parasitised. Thus, lower moisture contents did not appear to be a major factor in causing the low levels of parasitism.

Discussion

The crucial outcome of this study is that parasitism rates resulting from artificial inoculations using the original procedures were substantially less compared to the original results by Bedding and Akhurst (1974). Therefore, the changes to the inoculation procedure (Table 2) are not considered to be the major cause of the low rates of parasitism from the inoculations from 1985 to 1988 (Table 1).

Finding the cause of the low rates of parasitism is still of major importance. A hypothesis of the cause is that a genetic change in the nematode has made it less infective (i.e., the nematode has become less sensitive to stimuli that activates the change from the fungus-feeding form to the parasitic form). The nematode has been subcultured in the laboratory since 1967 (R. A. Bedding, pers. comm.). In laboratory cultures, only nematodes that remain in the fungus-feeding cycle are perpetuated (i.e., nematodes that change to the parasitic cycle are not able to reproduce in the cultures). Therefore, nematodes in laboratory cultures are under a selection factor that is diametrically opposed to a selection factor in nature (i.e., the parasitic cycle of the nematode must be activated each sirex generation in nature, so nematodes can be carried by sirex to a new supply of fungus). Bedding (1972) observed that one strain of *B. siricidicola* no longer produced infective females in plate cultures after several years of subculturing in the laboratory. Thus, further studies are needed to test this hypothesis of reduced infective capacity of nematodes repeatedly subcultured in the laboratory.

If nematodes from the laboratory cultures are less infectious than the "naturally occurring" type, the balance between sirex and nematode populations may be altered, and economically significant levels of tree mortality may occur before populations of the "laboratory" type of nematode reach parasitism rates that regulate sirex populations. Thus, these proposed studies are necessary to ensure that this biological control system is returned to an effective standard.

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Table 3. Percentage of sirex parasitised by *Beddingia siricidicola* (mean and standard deviation) from the paired inoculated and uninoculated logs.

			Percentage of sirex parasitised			
			Inoculated		Uninoculated	
GC[1]	Hammer Type	Rep.[2]	Mean	s.d.	mean	s.d.
12%	Gould	6	60.7	40.0	40.5	46.3
12%	SWAT	8	47.5	28.5	52.5	30.9
9%	SWAT	7	75.9	20.1	42.8	40.8
6%	SWAT	6	61.1	40.4	56.1	44.7
3%	SWAT	7	39.0	41.3	36.0	45.1

[1] Gelatin concentration of nematode carrier.
[2] Number of replicates with 10 or more sirex dissected from each of the paired inoculated and uninoculated logs.

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