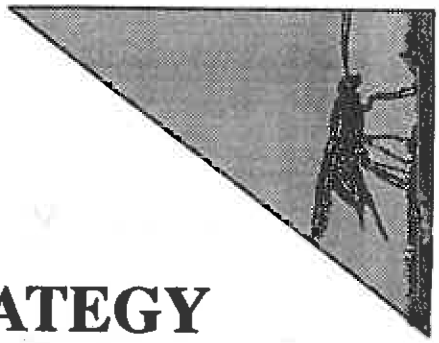

NATIONAL SIREX CONTROL STRATEGY

OPERATIONS WORKSHEETS

To be read in conjunction with the National Strategy for the Control of *Sirex noctilio* in Australia

CONTENTS

The National Sirex Co-ordination Committee (NSCC) has developed these worksheets in order to ensure a consistent and high standard for the various operations which form their National Sirex Control Strategy.

The NSCC reports to the Australian Forestry Council and includes representatives of state forest services and private plantation growers.

Produced by the National Sirex Co-ordination Committee August 1991

NATIONAL SIREX CONTROL STRATEGY

Summary of major tasks necessary for effective biological control of sirex woodwasp.

Before sirex detected

- Quarantine
- Training of staff
- Sirex survey and trap tree establishment
- Thinning

After sirex detected

- Map and monitor tree mortality
- Thinning
- Establish trap trees and inoculate with nematodes
- Determine establishment and effectiveness of biological control agents
- Release parasitoids

After biological control agents are well established

- Monitor sirex in susceptible plantations by survey or trap trees
- Release further biological control agents if required
- Evaluate plantations for nematode parasitism of sirex and parasitoid presence

OVERVIEW OF SIREX CONTROL

INTRODUCTION

Sirex noctilio, a native of Europe, is the only one of a large number of woodwasp species able to kill relatively healthy pine trees. One of the tree species most susceptible to this insect, *Pinus radiata*, from California, was introduced into Australia during the nineteenth century and now forms the bulk of this country's 900,000 hectares of softwood plantation. Sirex was accidentally introduced into New Zealand and caused epidemic outbreaks during 1945-1949. It was discovered near Hobart during 1952 and then near Melbourne during 1961.

Since then, sirex has spread throughout Tasmania and Victoria, and as of 1991 has reached the Adelaide Hills in South Australia and Newcastle in New South Wales. The combination of a particularly susceptible tree species and a very damaging woodwasp, brought together in drought-prone Australia, is a recipe for disaster and indeed serious outbreaks have occurred in Tasmania, Victoria, and South Australia. If the control measures outlined in this document had been implemented, these outbreaks may have been averted.

The National Sirex Co-ordination Committee, a group of pest management scientists drawn from the forest services involved in growing pines, has developed a National Strategy for the control of *Sirex noctilio* in Australia, which was published in the Australian Forest Grower, Winter 1990. These worksheets supplement the National Strategy, providing more detail on the essential tasks necessary for effective control of sirex.

LIFE CYCLE

In Australia, sirex normally completes one generation per year (a small proportion of the population may take two years). Adults live for only a few days and emerge from December through to May, with the peak emergence between January and March. The female wasp drills her ovipositor into the outer sapwood of trees to lay eggs. At the same time she injects a symbiotic fungus (*Amylostereum areolatum*) and a toxic mucus which together cause the death of the tree. Sirex larvae feed on the fungus as they tunnel through the wood. Mature larvae pupate close to the bark surface the following summer and adults emerge about three weeks later.

Trees attacked by sirex begin to show conspicuous symptoms from April onwards. The entire crown wilts and turns light green to yellow then to coppery brown. Beads or dribbles of resin, resulting from wounds during egg laying, may be visible on the bark. As the fungus grows from the point of oviposition, fungal stains appear in the cambium as long, narrow, brown bands along the grain, and eventually the fungus permeates every part of the tree. Larvae, galleries, or exit holes provide conclusive evidence of successful sirex attack. After a tree has been killed, the wood degrades rapidly and, if salvage is feasible, it should be done within six months of sirex attack.

CONTROL TECHNIQUES

Pinus species other than *P. radiata* are also susceptible to sirex attack. The most susceptible plantations are generally 10-25 year old and unthinned. Trees under stress (e.g. due to drought conditions) or injured (e.g. by wind, fire, or during logging) appear to be more susceptible to attack. Therefore, a major preventative measure is to maintain vigorous stand growth by timely thinning and by protection from fire, pests and diseases which may stress and weaken trees.

Two kinds of biological agents are used to control sirex. The parasitic nematode, *Deladenus siricidicola*, is of greatest importance. This nematode has an extraordinary life cycle which enables it to breed in vast numbers throughout the tree while feeding on the sirex-introduced fungus. When it detects a sirex larva it develops a parasitic phase, enters the larva, and begins reproduction when its host pupates. Nematode juveniles sterilise the adult female sirex by entering all her eggs. When nematode-infected sirex emerge and attack other trees, they transmit packets of nematodes instead of fertile eggs. Once a significant proportion of the sirex population becomes infected with the nematode (levels can approach 100%), the sirex population will collapse.

A number of parasitic wasps (parasitoids) have been imported and released for sirex control. *Ibalia* species lay their eggs down the drill holes in the tree made when the sirex female laid her eggs, and into the developing sirex eggs, eventually killing the sirex larvae from the inside. *Rhyssa, Megarhyssa* and

Schlettererius species drill deep into the wood to locate, paralyse and then lay their eggs on sirex larvae. The parasitoid larvae consume and kill the sirex larvae from the outside. The combined activity of these four parasitoid wasps does not usually kill more than 40% of a sirex population, and therefore parasitoid activity alone is not considered sufficient to prevent sirex from reaching outbreak levels.

CONTROL RECOMMENDATIONS

(A summary of these recommendations is located inside the front cover.)

- **Before sirex is detected in a region:**

- Consider whether quarantine measures will be effective and economically justified, and invoke where appropriate.

- Train forest, logging, and sawmill personnel to recognize sirex symptoms (with annual refresher sessions), and promote vigilant forest surveillance.

- Establish trap tree plots to detect whether sirex is present in susceptible plantations (i.e. 10-25 years old and more than two years past the prescribed thinning age), near mills, major transportation routes, and the leading edge of expected natural sirex dispersal. The number of plots should be proportional to the risk of sirex introduction. (Refer to Worksheet 2 for further detail.)

- Review the status of plantation thinning and comply with the optimum thinning guide for first and second thinnings.

- **Annual program once sirex is detected:**

- Map sirex distribution within the region (from forest surveillance and trap tree data).

- Estimate sirex-associated tree mortality in selected compartments by ground surveys along transects.

- Review the status of plantation thinning and comply with the optimum thinning guide for first and second thinnings.

- Establish trap tree plots during November/December, prior to sirex emergence, for later inoculation with nematodes. Select a plot density of at least one plot per 50 ha of susceptible plantation within the sirex distribution. Inoculate the sirex-attacked trap trees with nematodes during May-July. Naturally attacked trees may also be used for this process where infestation levels are severe.

- Release parasitoids in compartments where sirex has been detected; record and map the pertinent information (compartment identification, species, number of males and females released, and date).

- Determine the percentage of sirex infected with nematodes, and population levels of each parasitoid species by caging logs from sirex infested trees struck in specific compartments and assessing emergent wasps. Assessment of naturally struck trees will provide information on background levels of biological control agents, and assessment of inoculated trees will provide information on the effectiveness of the inoculations done.

- Review data and reports of the sirex control program from the current and previous year, and plan a work schedule for the following year.

- **After the biological control agents are well established in a region and the sirex population has declined:**

- Select plantations for sampling that are 10-12 years old and geographically isolated from current populations of the biological control agents.

- Establish trap tree plots in these plantations to confirm the presence of sirex.

- Evaluate logs from these trap trees to determine the percentage of sirex infected with nematodes and the presence of parasitoids.

- Make further releases if the levels of biological control agents are inadequate.

The attached worksheets provide the techniques and prescriptions necessary for successful implementation of the sirex control program in the field.

Work Sheet 1

MONITORING SIREX POPULATIONS

OBJECTIVE
To determine the distribution and the level of infestation of sirex throughout a given area of plantation, and to detect changes in population levels from year to year. Results will enable planning and implementation of appropriate management responses.

KEY FACTORS
1. Early detection of sirex in plantations.

2. Capacity to survey often large areas of plantation quickly, economically and reliably (aerial survey).

3. Accurate, reproducible sampling of specific blocks (ground survey).

4. Survey timing.

TRAP TREES
Trap tree plots (see Sirex Worksheet 2) will assist in the detection of sirex at the earliest stages of sirex establishment in a plantation, often 2-3 years before dying trees become evident.

In those plantations where sirex is suspected but has not been detected, trap trees should be established at a sufficient number of locations to provide a representative sample of the area. Plots should be close to all-weather roads for ease of access. These plots also provide initial release sites for nematodes if required.

Note: Trap trees are usually only receptive for one sirex flight season. New plots are required annually.

AERIAL SURVEY
Aerial surveys are most useful for covering large areas and stratifying plantation compartments by their infestation levels. Later verification must be made by detailed ground survey.

Survey from fixed-wing aircraft does not appear to accurately quantify tree mortality, particularly at the critical levels below 5%. Use of a helicopter provides the advantages of slower speeds, and greater manoeuvrability (ability to hover or land as necessary).

Aerial survey for early detection
Early detection of sirex is best achieved with trap trees (see above). In the absence of trap trees, aerial survey can be used to locate suspect trees or areas for further investigation. Any dead or dying trees should be noted. In the early stages of infestation, sirex are most likely to kill suppressed trees, not readily visible from the air. Aerial survey without follow-up ground survey can thus be misleading. Clumps of dead trees are unlikely to be caused by, but may contain some, sirex and should be investigated.

Aerial survey as part of a control program
In areas where sirex is known to be established, aerial survey can be used to determine the distribution and relative intensity of recent sirex-associated tree deaths. Stratification of a plantation by compartment for later ground survey sampling is the objective. Stratification should be made at the following levels to assign infestation classes:

<1% tree deaths
1-5% tree deaths
>5% tree deaths

GROUND SURVEY
Ground survey is used to sample representative areas (compartments) and to verify the reliability of aerial estimates. It provides a more accurate estimate of the proportion of trees killed by sirex within a stand and will result in a more accurate definition of infestation levels as outlined in Table 1 (Worksheet 2). Sample compartments should be selected using the following criteria:

1. Susceptibility to sirex attack - age (>10 years), likely time to first thinning.

2. Infestation class from aerial survey.

3. Representativeness of the compartment to its surrounds.

4. Sirex history of the area.

The number of compartments sampled will depend upon logistics but should be sufficient to provide full coverage of plantation variability.

The surveys should be carried out by appropriately trained personnel. The method involves sampling either on fixed or annually selected representative transects. The method described below has been found to provide reliable results using annually selected transects.

Method

All trees within two rows are counted while walking between those rows through the compartment. A hand-held counter is essential for rapid and accurate counts. Dead or dying trees are recorded on a Sirex Ground Survey Record Sheet (attached) and tallied according to the observed cause of death and the tree's dominance classification (dominant, codominant, suppressed). These forms can be processed later onto summary sheets or recorded on a suitable computer spread sheet or data base system. The data are usually processed to provide % mortality (of original stocking) attributable to sirex on a compartmental or age-class basis.

The composition of survey crews is flexible and may vary from one person in low infestation level stands, to a three-man crew in heavily infested stands. A three-man crew (two observers and a recorder) has the advantage of covering a broader band of trees (4-6 rows) within a single transect. (The recorder could also act as an observer in low infestation stands.)

The number of trees required to be surveyed in order to obtain an accurate assessment of sirex infestation within a compartment will vary but should take into account variations of aspect, topography and aerially observed distribution of sirex-killed trees within a compartment. Transects accounting for 2-3% of the total trees in the compartment should give reliable results. If using fixed transects, a paint mark on dead trees will improve the speed and effectiveness of subsequent surveys.

SURVEY TIMING

Ground and/or aerial surveys should be carried out from mid-May through to the end of July. Symptoms of recent infestation are usually most apparent and easiest to confirm during this period. Earlier surveys run the risk of missing trees attacked later in the flight season, but will provide early information where inoculation decisions need to be made. Later surveys (after July) may find symptoms of recent attack more difficult to detect, but will give the best estimate of total season mortality.

Work Sheet 1

SIREX GROUND SURVEY RECORD SHEET

PLANTATION BLOCK .... DATE ....

COMPARTMENT .... AREA ....

YEAR PLANTED .... TRANSECT NUMBER ....

INITIAL STOCKING (no./ha) ....

Dead / dying trees due to sirex						Dead from other causes		
Current year deaths. (Coppery needle colour, wilting, resin beading, cambial staining)			Old deaths (Emergence holes present)					
* Dominant	Codominant	Suppressed	Dominant	Codominant	Suppressed	Dominant	Codominant	Suppressed
Total			Total			Total		

* Note that observations need not be subdivided into dominance classes as above if not required.

Remarks:

Total trees surveyed:

	Current year sirex deaths	Old sirex deaths	Other deaths	TOTAL
# dead /ha				
Mortality (%)				

Work Sheet 2

TRAP TREE ESTABLISHMENT

OBJECTIVE
To establish a network of trees which will be attractive to sirex attack during the peak of the flight season, in order to:
1. Detect the presence of sirex in an area;
2. Provide an efficient means of introducing sirex biological control agents; and/or
3. Monitor population levels of sirex and its biological control agents where sirex is already established but population levels are low.

KEY FACTORS
1. Location of trap tree plots in accessible locations within plantations susceptible to sirex attack.
2. Technique used to poison the trap trees.
3. Timing of trap tree poisoning to ensure that the trees are attractive to sirex during the peak emergence time (summer months).

SITE SELECTION
Trap trees are of greatest value in areas where sirex levels are low or where naturally struck trees are difficult to locate or access.

Trap trees should be located in plantations susceptible to sirex attack (unthinned plantations 10-25 years old, particularly where thinning has been delayed by 2 or more years, or where plantations have been damaged by fire, disease or other agency).

Size of trap tree plots will vary depending on the purpose of the plot:
- Detection plots, in areas where sirex has not been found should consist of 5 trees per plot.
- Inoculation plots in areas where sirex has been detected should consist of 10 trees per plot.
- Monitoring plots in areas where sirex populations have collapsed should consist of 5 trees per plot.

Table 1 Selection of trap tree plot density

Annual tree deaths	Infestation level	Required plot density
<1%	Light	2 plots /100 ha
1-3%	Critical	4 plots/100 ha
>3%	Severe	* 4 plots/100 ha + inoculate 10-20% of naturally attacked trees

** where nematode infection levels are below 10%.*

The distribution of trap tree plots is determined by considering the infestation level of sirex in the plantation (refer Table 1). Sites should be accessible from all-weather roads to ensure that assessment during winter is not hindered.

Plots may be set up as line plots or as a group of scattered trees. Select trees of approximately 15-20 cm diameter at breast height over bark (DBHOB), preferably with poor form, in order to minimise wastage of quality products. Issues such as ease of felling and minimisation of damage to the surrounding stand should be considered. Each tree should be distinctly marked, and numbered for later identification.

TREE INJECTION
Sirex are attracted to pines under stress. Trap trees are injected with herbicide which causes them to die slowly, making the trees attractive to sirex over most of the flight season. The optimum time for preparing trap trees is 6-8 weeks prior to the sirex emergence period of December to May.

Trees are prepared by trimming the branches to make the butt accessible. Herbicide is injected slowly into the sapwood at a convenient height above the butt of the tree, <1 m from the ground. Access to the sapwood can be via drilled holes, approximately 5 cm deep and 1 cm in diameter using equipment such as a brace and bit, cordless drill, air drill, or post hole borer attached to a chainsaw motor. Alternatively a tree injector such as an INVjector® could be used to inject the herbicide, or the tree could be fringed with a pruning axe after which herbicide is injected using a syringe, a Velpar Spot Gun® or a Phillips Auto Vax®.

It is important that the herbicide be injected into the sapwood and not into the bark of the tree.

Suitable herbicides are:

- dicamba (200 g/kg) sold as "Banvel 200"® by Sandoz Australia Pty Ltd, "Dicamba 200"® by Consolidated Fertilisers Ltd or Davison Industries Pty Ltd, and "Dicamba"® by Rhone Phoulenc; or

- triclopyr (100 g/kg) sold as "Triclopyr amine"® or "Tordon"® by Dow Chemical.

The recommended injection rate for all these chemicals is 2 ml of undiluted chemical applied every 10 cm around the stem.

ASSESSING RESULTS

Trees should be examined during the following autumn to early winter for signs of dieback due to the herbicide and for symptoms of sirex attack. The most distinguishable symptoms of sirex attack, especially during winter, are the wilting and coppery brown colour of needles, presence of resin beads and /or dribbles, and narrow bands of orange-brown fungal staining on the cambium layer just beneath the bark, mainly along the grain of the timber.

These bands of stain from the sirex fungus *Amylostereum areolatum* normally give a good indication of the presence of sirex but are sometimes difficult to detect.

Fine, powdery, frass-packed galleries will also be present upon cross-cutting the tree.

Trees that die should be felled and prepared for inoculation or monitoring. Logs for inoculation should not be cut into short lengths as excessive drying may then occur. Logs for monitoring should be cut and the ends coated with a fungicide to prevent infection by blue stain. After several hours cut ends should be sealed with either beeswax or mastic to restrict moisture loss. The logs can be placed on blocks to prevent ground contact. This may delay wood decay and blue stain development and allow sirex to emerge around the entire circumference of the tree.

If the tree appears healthy and has survived the herbicide treatment, there is no need to cut it down as it unlikely to have been attacked by sirex.

The attached Trap Tree Plot Data Sheet should be used to record all operations on the plots including poisoning, assessment of poisoning success, felling, inoculation and follow-up assessment of sirex emergence holes 18 months after herbicide treatment.

Work Sheet 2

TRAP TREE PLOT DATA SHEET

TRAP TREE PLOT NUMBER ....

PLANTATION BLOCK.................................
COMPARTMENT..
YEAR PLANTED ....

PLOT TYPE: Line / Scattered trees / ....
Detection / Inoculation / Monitoring
5 tree / 10 tree / ....

HISTORY OF SIREX DAMAGE:

YEAR	....	....
TREES RECENTLY KILLED		
- NUMBER PER HA	....	....
- % ORIGINAL STOCKING	....	....

LAST THINNING:
YEAR
TYPE UT / WT / T1 / T2 / DT1 / DT2 /

COMPARTMENT SKETCH & plot location

	POISON DATE	ASSESSMENT OF POISON SUCCESS *			FELL	SIREX STAIN	INOCU-LATION	EMERGENCE HOLES Estimate total/tree **
DATE								
CREW								
TREE 1		Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	
2		Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	
3		Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	
4		Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	
5		Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	
6		Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	
7		Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	
8		Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	
9		Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	
10		Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	

* Poison success is Y if any sign of the tree being unhealthy.
** Representative sample of trees only need be assessed.

COMMENTS:

Note: This data sheet is to be used in conjunction with Sirex Worksheet 2.

NOTES ON COMPLETING THE TRAP TREE PLOT DATA SHEET

Thinning Operations Key

UT	Unthinned
WT	Waste thinning
T1	First commercial thinning
T2	Second commercial thinning
DT1	Delayed first thinning
DT2	Delayed second thinning

Complete the relevant tree data at each visit to the trap tree plot.

NEMATODE HANDLING AND INOCULATION

OBJECTIVE
To inoculate sirex infested trees with the nematode *Deladenus siricidicola*, so that >90% of emerging sirex are infected with the nematode.

KEY FACTORS
1. Fresh nematodes, transported and stored under optimal conditions.
2. Preparation of only sufficient flummery for one day's use.
3. Preparation of consistent flummery mixes containing 12% gelatin.
4. Inoculation with approximately 2,000 nematodes per inoculation hole (greater numbers of nematodes result in smaller sized, less useful parasitised sirex).
5. Correct storage, transport and handling of nematode flummery mixes.
6. Inoculation of sirex-infested trees at the optimum time of the year.
7. Clean cutting of tracheids to allow nematode movement into the wood, using a very sharp inoculation punch.

NEMATODE HANDLING
Nematodes are transported in 75 ml phials containing 1 million nematodes in 20 ml of water. These should be packed in insulated boxes with freezer blocks included and kept between 5°C- 15°C at all times. The optimum storage temperature is 5°C. At higher temperatures, less oxygen is dissolved in the water, and the nematodes use more oxygen thus increasing the probability of nematode mortality.

Nematodes should be used as soon as possible after they are received, however they can be kept for up to 5 days from despatch under optimum conditions. Samples of nematodes should be checked under a microscope prior to mixing to ensure that they are alive; under magnification healthy nematodes will appear active and curled, whereas dead or unhealthy nematodes will appear straight and lifeless.

When stored in a refrigerator, a maximum/minimum thermometer should be included to ensure the acceptable temperature range is maintained.

FLUMMERY MIXING
The aim is to produce a 12% gelatin mix that equates to 2,000 nematodes per inoculation hole.

Recipe

Using a large electrical mixer (e.g. Kenwood Major with 6 L bowl):
1. Add 550 mL of **boiling** water to the mixer bowl.
2. Mix in 210 g of gelatin, first stirring, and then using the mixer on a medium speed setting for 1 minute (any longer will risk cooling and premature setting).
3. At the same setting, slowly pour in 1 L of ice-cold water (1-2°C), and increase the speed to maximum for 2.5 minutes.
4. Bulk together 9 phials of nematodes (9 million in 180 mL).
5. Before adding nematodes, ensure mix is below 20°C to avoid nematode death. Reduce the speed to medium, and pour in nematodes. Add several drops of food colouring dye to indicate uniformity of mixture. (A different dye colour for each day of the week helps to ensure mixtures are used on the day of preparation.) Continue mixing for a further 1 minute until the dye is evenly distributed.

A mix of this volume is sufficient for about 4,500 inoculation holes (50-100 trees). Reduce proportionately where fewer trees are to be treated.

FLUMMERY TRANSPORT AND STORAGE
This mix can then be dispensed into plastic bags for transport. Transport and store this mixture at a temperature as near to 5° C as is practical.

Use the flummery as quickly as possible, and definitely within 36 hours of mixing. (Do not store mix in dispensers.)

The flummery can be dispensed using either sealant guns or 50 ml syringes. (Sauce bottles previously used are not suitable for a 12%

flummery.) Both of these need a narrow tip to fit in the inoculation hole, and the gun can have a poprivet placed in the removable tip with the stem outwards, to act as a valve to stop dribbling. To transfer flummery from plastic bags, snip a corner of the bag and squeeze flummery out into the cartridge or syringe.

INOCULATION PROCESS

Timing

Optimum inoculation time is considered to be during the period May through July. The limitations are that prior to May many of the dying naturally struck trees aren't obvious, while by August the moisture content of dying trees is likely to be too low to ensure success. (Moisture content of >50% is considered preferable.)

Tree Selection

Trees for inoculation (trap trees or naturally struck trees) should be current year deaths, infested with sirex larvae. Symptoms are:

- wilted foliage, yellow to coppery brown in colour,
- resin beads on the outer bark resulting from wounds during egg laying,
- orange-brown fungal staining of the cambium when the bark is removed,
- fine, powdery, frass-packed galleries present upon cross-cutting the tree.

If a tree has sirex exit holes, it has died in a previous year and should not be inoculated.

If nematode levels have not been assessed in an area before, some trees should be assessed by examining woodchips (Worksheet 4) to estimate nematode levels prior to inoculation.

See Table 2, Worksheet 4 for recommendations on inoculation intensity.

Inoculation

The trees for inoculation should be felled, and trimmed of branches on the top side to facilitate inoculation.

Materials

- Rebound inoculation hammer with punches (may be purchased from: Sirex Officer, Keith Turnbull Research Institute, PO Box 48, Frankston, VIC 3199).
- Nematode flummery mixture.
- Nematode flummery dispenser - sealant gun or syringe.

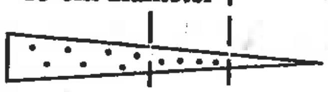

Fig 1. Location of inoculation holes in tree.

Method

Inoculation holes approximately 10 mm deep are made using the rebound hammer. Hole spacing should be:

- log diameter over bark >15 cm - 2 rows of staggered holes 30 cm apart along the log in about the 10 and 2 o'clock positions.
- log diameter over bark <15 cm - 1 row of holes 30 cm apart along the top of the log down to about 5 cm in diameter.

There is no need to inoculate in the deep fissured bark at the butt of the tree, as sirex is less likely to have oviposited in this part of the tree.

The tip of the flummery dispenser is inserted into the base of the inoculation hole and withdrawn as inoculum is squeezed into it. Once filled, the flummery is compacted by pressing with a finger or thumb. This compaction is very important as it ensures complete contact between the flummery and the inside of the hole so that nematodes can move into the wood easily.

Inoculation on rainy days and days >20°C should be avoided because:

- Rain may wash flummery out of holes.
- Warm temperatures dry out the flummery, leading to death of the nematodes before they enter the wood.

The cutting surface of the punch should be kept sharp and even at all times using a chainsaw file so that tracheids are cut cleanly, permitting nematodes to readily move into the wood from the flummery. If the punch goes dull, replace it so that it can be sharpened. Rebound rubbers should be replaced regularly so that rebound is maintained.

Lubrication of the inside of the punch (especially overnight) maintains cutting performance and ejection of wood cores.

Tree inoculation is usually carried out by teams of 2 or 3 people. One person punches holes while the other(s) follow behind inoculating. An average of 15-25 trees can be felled and inoculated per person in a day, depending on travelling time, tree size and terrain.

Calculation of 12% gelatin mix

210 g gelatin is mixed with 1730 mL of water.
(550 ml + 1,000 ml + 180 ml)
Weight gelatin/weight water = 210/1730 = 12%.

EVALUATION OF SIREX BIOLOGICAL CONTROL AGENTS

OBJECTIVE

To determine establishment, distribution and relative abundance of biological control agents in specific geographic localities.

KEY FACTORS

1. Evaluate effectiveness of specific release operations for establishment.

2. Evaluation should relate to specific localities to determine dispersal and estimate distribution.

3. Results should determine future release programs.

OVERVIEW

Nematodes

For a rapid assessment of nematode presence at the time of tree death, woodchips from dying trees can be extracted and checked for nematode presence. This will give an indication of the distribution, and hence activity of nematodes in an area. The woodchip sampling technique is outlined over the page.

The main method of assessing nematode levels is to dissect sirex emerging from caged logs and examine for nematodes under a 40X magnification. The aim is to determine the proportion of sirex infected with the nematodes, expressed as % infected sirex.

It is important to determine the "background" level of nematodes in a sirex population and/or to determine the effectiveness of sirex inoculation. Comparing nematode levels from inoculated trees with non-inoculated trees will indicate the effectiveness of inoculation, and therefore the efficiency of the control operation.

Logs should be cut from sirex-infested trees and the ends coated with a fungicide (e.g. Captan) to prevent infection by bluestain. Several hours later the cut ends are then sealed with either beeswax or mastic to reduce moisture loss (eg. Caltex Timber Sealer). Logs are placed in cages prior to sirex emergence in summer. (See 'Techniques' over the page.) The emergent sirex are collected and assessed from December through to May.

Both male and female sirex can be assessed for nematodes by cutting the abdomen off and inspecting the squashed abdomen in water for nematodes, under a microscope. Where 100 insects have been examined from any cage, only a sub-sample (e.g. 10%) of further emergents need be examined for nematodes.

A stereo microscope with variable magnification is necessary to detect the nematodes (with lighting from a dark-field base if available). If nematodes are numerous they will be visible at 10X magnification, but 40X or 50X magnification may be necessary to detect nematodes at low levels.

The results should be summarised by locality and not pooled for the whole region, as management decisions need to be made at the locality level.

The number of areas sampled must be sufficient to allow decisions on insect control to be made with confidence. For example, for nematode assessment in South Australia, 25 uninoculated trees are sampled in each chosen locality (1 x 0.8 m log per tree; 5 trees per compartment and 5 compartments per locality). Sample logs are taken from the "mid stratum point" (defined as halfway between the butt and the 5 cm diameter point at the tree top).

Parasitoids

The aim of parasitoid assessment is to determine the presence and relative abundance of sirex parasitoids.

Ibalia has a similar emergence period to that of sirex (summer/autumn) as it parasitises the sirex eggs, and therefore data for ibalia can be assessed using the same emergence cages and logs described for collecting sirex for nematode evaluation (above).

The other parasitoids attack sirex larvae, and therefore emerge in spring, 6 months after non-parasitised sirex have emerged from the same log, and just prior to the next sirex flight season. They are also assessed using emergence cages, however it is preferable for sample logs to be collected in the September after sirex emergence.

Trees to be sampled for parasitoids other than ibalia should be marked in the year they die to ensure that samples taken in September are from trees killed the previous year. These trees will have sirex exit holes in the stem.

Background nematode level from drum samples (% infection)	Recommended inoculation level (% of sirex infested-trees)
0%	20%
1-5%	10%
5-10%	5%
>10%	0%

Table 2. Recommended inoculation levels where annual sirex-associated tree deaths exceed 0.5%

Where detection of parasitoids is the objective, logs should be selected for maximum number of exit holes. Where a representative population level is needed, logs should be cut from half way up the tree.

TECHNIQUES

Emergence Cages

The principle is to place short logs (0.8-1.5 m length) from sirex infested trees into dark cages (ventilated 200 L drums or emergence rooms). Emerging insects are attracted to a light source where they are captured. Sirex are then dissected to assess for nematode presence.

If using 200 L drums, the interior of the drums should be non-toxic to ensure good insect survival and to minimize rust problems. Insects can be collected via a detachable bottle at the light source or through a hand hole in mesh covering the top of the drum. Drums must be kept in the shade to prevent overheating.

Drums can be stored horizontal or vertical, but in both cases regular (weekly) inspections of the entire drum are necessary to collect uncaptured insects. Horizontal drums are more space efficient, however they may distort at the opening due to drums on top, making opening and closing for full inspection difficult. Use of bracing and spacers betweeen rows of drums will help prevent this problem.

Emergence rooms should be dry and well ventilated. Logs may be stacked horizontally or vertically in the room. Cages should be inspected regularly during the emergence season, and all emerging insects recorded by species, sex and number.

Woodchip Sampling

Chip sampling, carried out in May or June, involves soaking a single chip of wood in water and then examining the water for nematodes.

The chip is obtained by removing an 8 cm square of bark to expose the sapwood. Two deep cross-grain axe cuts are made 6 cm apart, with the second parallel to the first, but at an angle into the tree so that the chip pops out. Preferred chip size is approximately 6 cm square and 1-1.5 cm thick.

Samples should be taken from several places in each tree (e.g. lower, middle, upper and top) and analysed separately.

Any bark, cambium or sirex fungus residues should be removed from the chip, which is then placed immediately into a rinsed, labelled tub containing 200 mL water. Tubs, water and chips should be kept away from direct sunlight to avoid overheating.

After allowing the chip to soak for 24-36 hours excess water should be decanted off without agitating the mix, leaving any nematodes concentrated in about 20 mL of liquid at the bottom. Place the remaining 20 mL in a petri dish and examine under 40X magnification for presence of nematodes.

EVALUATION OF RESULTS

Nematode levels

Information on the level of nematode infection is vital for predicting future sirex levels and planning future control programs, as nematodes are the prime biological control agent.

The nematode level from cage samples reflects the nematode status of trees killed the previous year. Therefore, when deciding on future inoculation an allowance must be made for the increase in nematode infection levels in the trees that are killed in the current year. Table 2 provides a guide to future inoculation requirements (trap & naturally struck trees) in locations where annual sirex-associated tree death exceeds 0.5%.

Sirex levels

Where more than 25 trees have been sampled in a locality, the average number of emerging sirex gives a reliable indication of the sirex abundance in that locality. This information should be assessed with tree mortality data and nematode levels to predict future sirex levels and plan the control action that will be required.

Parasitoid levels

Parasitoid presence indicates that the particular species is established in the area. This and the knowledge of previous releases of the parasitoid can indicate the distribution of the parasitoid. Data should be used to map the expected distribution and select sites for further releases.

REARING OF SIREX PARASITOIDS

OBJECTIVE

The object of this program is to breed sufficient sirex parasitoids to maintain viable breeding populations and supply additional insects for release to the field.

KEY FACTORS

1. Knowledge of distribution of parasitoid species in the field to enable field collection of larvae.
2. Selection of appropriate logs containing parasitoids for collection.
3. Availability of logs containing high numbers of average to large sirex larvae.
4. Maintenance of a wide gene pool within insectary populations.

RHYSSINE PARASITOIDS

Field collection of parasitoids

Logs should be collected during June-August from trees killed by sirex in the previous calendar year, in localities where parasitoids are known to be established.

Trees selected for felling should have significant numbers of sirex emergence holes as evidence that sirex larvae were present in the log during the previous season. After felling, logs are cut from where exit holes are present. Evidence of parasitoid pupae may be found if logs are cross-cut and split. Logs should be1.5-1.8 m long, and ideally of 15-20 cm diameter. Cut ends are coated with fungicide (e.g. Captan) to prevent infection by bluestain; and then several hours later sealed with mastic or beeswax to reduce moisture loss.

Parasitoid emergence

Sample logs are placed in an insectary room or into emergence drums (see Worksheet 4). Logs which are to be caged in drums are cut to 0.8 m length. In an insectary emergence room the 1.5 m logs can be stacked horizontally (cross-hatch), allowing plenty of space between each log for air circulation and insect emergence.

In southern Australia emergence of *Rhyssa persuasoria* usually begins in early September, with *Megarhyssa spp.* commencing late September, and *Rhyssa hoferi* and *Sclettererius cinctipes* in late October. All finish emerging 10-12 weeks later; however, some short life cycle *Megarhyssa spp.* may emerge mid-December through to February. These emergence times may be brought forward in warmer, drier areas.

Males make up a large proportion among the early emergents before female numbers increase. These early emergents are collected, allowed to feed on a solution of honey and water (1:5) and then kept at 5-7°C to slow their metabolism. It is advisable to place the containers of insects at room temperature every third day and allow the insects to feed. They can be stored in this manner for up to 10 days without detrimental effects as long as high humidity is maintained.

Rearing techniques

The rearing rooms are fitted with racks allowing sirex infested logs to be held vertically and providing ample light and air circulation.

Logs for the rearing room are collected from August/September (Victoria) through to October (Tasmania) from trees infested in the field with sirex. These logs should be 10-20cm in diameter, have no emergence holes prior to collection, andbe prepared as for logs under 'Field collection of parasitoids'. Alternatively logs from insectary sirex cultures may be used. In both cases, the logs should contain numerous, average to large sirex larvae. *Rhyssa* prefers the smaller logs, whilst *Megarhyssa* will favour the larger log sizes.

Each rearing room should contain an optimum ratio of two females per 1.5 m log. This avoids overcrowding which may cause disturbance of ovipositing females.

Males of both *Rhyssa* and *Megarhyssa* are not ready to mate for several days after emergence (Taylor 1967 a,b) and so should be allowed to mature for 2-3 days and then refrigerated. Once a suitable number of females has emerged (e.g. 5), stored mature males can be introduced at a male:female ratio of 2:1. Females and males should be allowed to stabilise to room temperature before release. They may be combined within small collecting containers for a few hours to promote mating.

Most female rhyssines can mate as soon as they emerge but do not oviposit until about a week after emergence (Taylor 1967 a,b). By keeping adults for a week and providing them with a honey solution as a food supplement and water, they will oviposit upon release in the field. Addition of a pollen source such as yeast autolysate may also have some value. For insectary culture one-week-old adults are transferred into rearing rooms set up with logs infested with sirex larvae (see Worksheet 6).

Parasitoids within these rooms should be supplied with a honey solution, water and a pollen source to increase their longevity (Hocking 1967), and thus improve the level of parasitism achieved.

After the parasitoid "flight" season (September-January), the rearing rooms remain dormant until the following September-January period, when the adult emergents are collected and used to establish or replenish field sites, or provide the next generation for insectary cultures. However, there can be some emergence of 'short life cycle' parasitoids in summer-autumn (e.g. up to 24% of *Megarhyssa nortoni* may emerge as short life cycle individuals, 2-3 months after oviposition).

If the sirex-infested logs used were from field collections, it is likely that some ibalia and sirex will emerge during the summer/autumn period.

IBALIA PARASITOIDS

Generally no breeding of ibalia parasitoids is necessary as these are widespread and can be easily retrieved from the field or from logs collected from the field for Rhyssine culture, if required.

To retrieve ibalia from the field, sirex infested logs should be collected by December prior to any insect emergence.

Ibalia parasitises sirex eggs and has a life cycle similar to that of sirex (summer-autumn emergence period). Rearing rooms need to be supplied with fresh logs into which sirex can oviposit, followed by ibalia.

Ibalia adults seem to seldom feed and rarely take water, however, water should always be provided in the insectary (Taylor 1967 a,b).

Early emergents may be stored, similarly to the rhyssines above. However, overcrowding should be avoided to prevent damage and premature death of parasitoids.

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BREEDING NEMATODE-FREE SIREX

OBJECTIVE

To establish and maintain a sirex population that is free of the parasitic nematode *Deladenus siricidicola.*

KEY FACTORS

1. Availability of nematode-free sirex in an insectary culture when such insects are no longer readily obtained in the field.

2. Maintaining a wide gene pool within the population.

3 Ensuring nematodes are excluded from the population.

INTRODUCTION

As the prime biological control agent of sirex, *Deladenus* nematodes have been widely and intensively used throughout sirex-infested plantations. This has drastically decreased the availability of sirex larvae, which are required for the purpose of breeding populations of parasitoids.

FIELD COLLECTION OF SIREX

To minimise the chance of collecting nematode-infected sirex, select a field site where inoculation has not previously been carried out, or where the intensity of inoculation has been low. Collect approximately 1.5-1.8 m pine logs from previously prepared trap trees, or from naturally attacked trees, between April and August. Trees for selection should be those infested by sirex during the previous summer and with the following characteristic symptoms:

1. Coppery brown wilted foliage.
2. The presence of sirex fungal staining on the cambium beneath the bark of the tree.
3. Small resin beads on the outer bark of the tree associated with sirex oviposition holes.
4. Fine, powdery, frass-packed galleries present upon cross-cutting the tree.

The presence of emergence holes would indicate that the tree had been killed in the previous year, and sirex larvae are no longer present. Such trees should therefore not be sampled.

As a check to determine whether nematodes are present and at what levels, the **"woodchip" test** can be applied to each tree (refer Worksheet 4).

Logs selected for transport to the insectary should have their ends treated with a fungicide to prevent infection by bluestain, and be sealed to retain moisture.

EMERGENCE CAGES

The selected logs should be placed in an insectary emergence room until sirex emergence occurs. They may be stacked either horizontally (cross-hatch) or vertically. Allow plenty of space between logs for light and air circulation, so as not to restrict insect emergence.

REARING CAGES

Rearing rooms are fitted with racks for holding freshly cut pine logs vertically. Allow plenty of space between logs for light and air circulation. The logs should be collected just prior to the sirex emergence period in order to maintain optimal log moisture levels for sirex fungus development. The logs should be about 1.5 m in length, and between 15 and 20 cm in diameter, and have their ends treated to restrict blue stain infection, and sealed to retain moisture. It may be advisable to allow logs to dry for several days in order to reach optimal moisture content for fungus development before cross-cutting and sealing the ends. Heavily furrowed thick bark, such as that near the butt of a tree should be avoided, to maximise the usable surface area for female sirex to oviposit.

Where possible subdivide the logs containing sirex larvae into several rearing cages to enhance protection against nematode infection of the population.

To ensure that emergent sirex are nematode-free, females should be tested prior to release into rearing rooms. Emerged females are collected and placed into small (approximately 60 x 120 cm) holding cages with freshly cut logs, and allowed to oviposit. Within 5 minutes of ovipositon, females are captured and their ovipositors dipped into drops of water, which are then examined under a microscope for the presence of nematodes. The nematode-free sirex females can then be released into the prepared rearing rooms. Both males and females should be supplied to the rearing room continuously throughout the flight period.

Screening for quality control
The female sirex in the rearing rooms should be collected soon after their death and examined more accurately by an experienced technician for the presence of nematodes. This is done by cutting off the abdomen and squeezing out its contents onto a Petri dish or microscope slide. Add a drop of water to the contents, spread with a probe, and then examine under 40X magnification. Sirex eggs are usually transparent, with nematodes readliy visible within. Many nematodes are also often visible within the body cavity. Live nematodes appear curled and should be moving, whereas dead nematodes appear straight, tapered and worm-like. If nematodes are detected then the cages from which they have been recovered must be treated as infested with nematodes. The progeny from such cages can be suitable for parasitoid breeding, but should not be used for sirex rearing in the subsequent season unless thoroughly tested and found free of nematodes.

Regular watering of logs with a knapsack spray during summer is advisable, especially if logs are of small diameter.

During winter, woodchip sampling of the rearing logs (Worksheet 4) is a wise precautionary measure. Any logs with nematodes should be discarded immediately from the sirex breeding program.

MAINTAINING THE POPULATION NEMATODE-FREE

Screening of female sirex must be repeated annually to ensure the purity of the population. It is also imperative that more than one rearing room be maintained in production at any time.