

# RESEARCH & DEVELOPMENT

## Sirex Wasp control with nematodes and trap trees

The Sirex Wasp (*Sirex noctilio*) is a major pest of Radiata Pine. Trees are attacked by the adult female during summer and autumn, and rapidly die as a result of injection into the sapwood of the symbiotic fungus *Amylostereum areolatum* and a phytotoxic mucus.

This issue of *Research & Development* concentrates on the control of the wasp with the parasitic nematode *Deladenus siricidicola*, which causes sterility among *Sirex* females. *Research & Development* No.6 contains information on the identification, biology and monitoring of the wasp.

### Background

The parasitic nematode is dimorphic: one form is free-living, able to feed on the symbiotic *Sirex* Fungus and reproduce over many generations. The other form is parasitic. When a free-living form approaches a *Sirex* larva, it becomes parasitic and invades it, eventually making the developing females sterile. Sterilised wasps will mate and oviposit normally, but their eggs can contain up to 200 juvenile nematodes and are therefore not viable. These nematodes can then parasitise the larval progeny of healthy *Sirex* females that have attacked the trees.

One technique used to introduce the nematode into *Sirex* populations was to locate infested trees, fell and trim them, and then inoculate the sapwood with the nematode. Another method was to culture infested billets in an insectary, inoculate them with nematodes, then distribute these billets throughout affected areas. These methods gave good results, but were very labour intensive and costly.

The use of 'trap trees' involves injecting selected trees with Dicamba herbicide, a much less labour intensive and expensive method that enables strategically placed and easily accessible groups of trees to come under stress early in the *Sirex* flight season. The stressed trees emit

volatile chemicals that attract *Sirex*. This technique eliminates the need to search for trees already attacked and so improves the efficiency of introducing nematodes into large plantations of susceptible pine. Trap tree sites are also useful for releases of parasitoid wasps and for monitoring early infestations of *Sirex*.

### Trap tree site selection

Trap trees should be positioned in *Sirex*-susceptible plantations areas that have been damaged by drought, wind, hail, fire or silvicultural treatments. Those in poor condition due to delayed thinning are highly susceptible. Each site should consist of about 10 trees positioned on a 1 km grid. Alternatively, select 10 trees per 50 to 100 ha of susceptible plantation. Sites should be near all-weather roads, for easy felling, inoculation, and relocation.

Select multi-stemmed, asymmetrical and suppressed trees, (in order to minimise wastage) and those of approximately 10 to 20 cm DBHOB. Trees can be either in a group or a row, to act as a focus of attraction for *Sirex*, and should be selected for ease of felling. Each tree needs to be distinctly marked.

### Tree injection

In Victoria the best time for preparing 'trap' trees by injecting them with herbicide is during November and December. Suitable herbicides are Dicamba (20% w/v), Fosamine (36% w/v) and Triclopyr (48% w/v). These have a slow rate of action on pine, making the trees attractive to *Sirex* over most of its flight season. Inject basally into the outer sapwood of the tree, using a tree injector such as an INVjector® or a pruning axe with a Velpar® spot gun. The recommended injection rate is 1 to 2 ml of undiluted chemical every 10 cm of stem circumference. If necessary, trim the lower branches to make the base of the selected trees accessible.

### **Tree inspection and felling**

Trees at all sites should be examined for the presence of *Sirex* during the following autumn to early winter.

The most distinguishable external symptoms of *Sirex* attack, especially during winter, are the narrow bands of slaty-grey or brown staining of the cambium layer, mainly along the grain of the timber. These bands are easily detected when the bark of suspected trees is removed. Other symptoms of *Sirex* attack include: needle chlorosis (yellowing) progressing to a copper-brown colour; sudden wilting of the old foliage, then of the current foliage; and numerous resin blobs and small resin runs on the stem.

Trees that prove positive for *Sirex* should be felled and trimmed to assist the inoculation process. The trees should be placed on blocks to raise the stems off the ground. This will delay wood decay and allow nematode-sterilised *Sirex* to emerge around the entire circumference of the tree.

### **Preparing an inoculum for injection**

The nematodes are usually dispatched from the Keith Turnbull Research Institute in 75 ml vials containing approximately 1 million nematodes in 20 ml of oxygenated water. Nematodes kept at 5°C can be stored for up to 1 week.

In Victoria, April to August is the best period for inoculation. The *Sirex*-symbiotic fungus, on which the nematodes will feed, is by then well distributed throughout the wood. The nematodes will have 4-7 months to locate their hosts and parasitise them.

The following procedure is recommended for preparing large quantities of gelatin foam inoculum (flummery); if you are using a smaller bowl, adjust measures accordingly:

1. To a 5 litre Kenwood Major® or similar mixing bowl, add 350 ml of hot water (about 90 to 100°C).
2. Sprinkle 90 g of household gelatine into the bowl, and stir the mixture at medium speed for at least two minutes until the gelatine is dissolved.
3. Add 1200 ml of iced water, prepared either by keeping quantities in a refrigerator, or by adding crushed ice to tap water, ensuring that no ice enters the mixing bowl.
4. Gradually increase to maximum speed. Beat until maximum volume is attained and until the mixture becomes firm and frothy (usually about one minute).
5. Reduce the mixing speed to medium and add 5 million nematodes (5 vials).

6. Add about 0.5 ml of food dye and continue at medium speed for approximately half to one minute to ensure uniform dispersal of the nematodes. When the dye is evenly distributed the nematodes can be assumed to be uniformly mixed.
7. Dispense the flummery mixture into plastic bags measuring approximately 25 x 40 cm and use as much of the mix from the whisk and bowl as practicable.
8. Rinse the whisk and bowl in hot water before starting the next mix.
9. Rinse the empty nematode vials and store for return to K.T.R.I. Clean all equipment thoroughly at the end of each day's mixing.

**The amount of inoculum stored overnight must be kept to a minimum, and should be used the following day. BOTH THE INOCULUM AND THE NEMATODE CONCENTRATE MUST BE STORED AT 5°C, AND MUST NEVER BE FROZEN. Use maximum/minimum thermometers in the refrigerators to ensure adequate temperature control.**

The inoculum must be stored and transported at between 5 and 15°C, in a portable cooler.

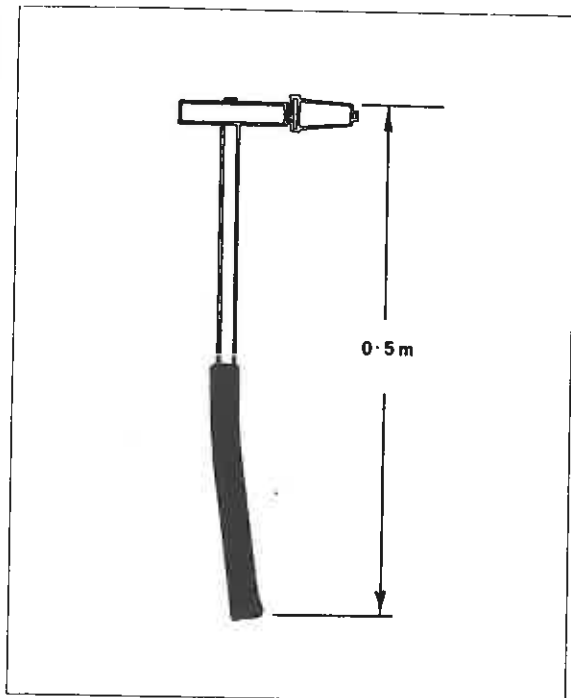
### **Field inoculation of trees**

For the inoculation of the nematode flummery into trees, the following equipment is used:

- An inoculating hammer consisting of a long handled hammer, with a modified wad punch attached (Figure 1). The hammer can be obtained from C.A.R. Industries, Mt Gambier, and the modified wad punches from K.T.R.I.
- An inoculum injector, consisting of a 250 ml plastic sauce dispenser, modified with a plastic pipette tip inserted into the nozzle.

Inoculation involves the following steps:

- Use the inoculating hammer to punch approximately 10 mm deep holes into the sapwood on either side of the tree. If the bark at the butt of the tree is thicker than the depth of the punch, chip the bark away to enable the punch to penetrate the sapwood.
- Maintain 10 to 15 cm between the alternate holes. The holes on each side of the tree should be 20 to 30 cm apart, with the holes on the other side being at the midpoint of this gap. Trees less than 15 cm need only a single row of holes 20-30 cm apart.
- Start inoculation at the butt of each tree and continue until the diameter is about 8 cm.



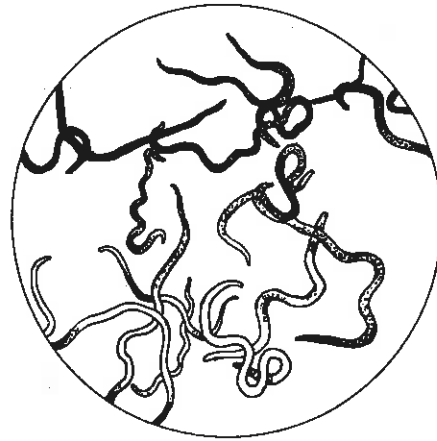
**Figure 1** This punch was originally designed by the late Mr. Vic Gould, Forests Commission of Victoria, and has been modified by staff of the Keith Turnbull Research Institute and Woods and Forests Department, S.A.

- Inject 0.5 to 1.0 ml of the nematode flummery into each hole (this is usually enough to fill it). Gradually withdraw the tip of the injector so that the flummery fills the hole from the bottom up.
- Seal the top of each hole by wiping it with the thumb or forefinger, thereby ensuring that there are no air gaps and that the flummery is against the sidewalls to help form a skin that stops it from drying out.

Tree inoculation is usually carried out by teams of 2 or 3 people. One person punches the holes and the others follow behind, to inject the holes with inoculum. Depending on the travelling time between sites, tree size, and the type of terrain, the average number of trees that can be inoculated is around 25 a day per person.

#### Evaluation

An annual evaluation of the effectiveness of the inoculation program is needed to highlight any shortcomings in the techniques used and to show whether enough nematodes have been released. In subsequent years nematode releases can be concentrated in areas where they have not become established. One way of evaluating the program is to examine infested trees for nematodes in the year after inoculation.



**Figure 2** Microscopic view of *Deladenus siricidicola* (40x magnification).

Nematodes can be detected by taking wood chips from the sapwood of infested trees throughout the affected area. The procedure is as follows:

- Remove a 5 cm square section of bark, to eliminate any nematodes that are not *Sirex*-specific from the bark and cambium;
- Make two deep axe cuts into this exposed area of sapwood so that a chip with a large cross-cut surface pops out;
- Place the sample chips from each tree into a separate, labelled plastic bag to prevent desiccation and nematode transfer;
- Soak the chips overnight, to induce the nematodes to float out of the wood into the water;
- Use a microscope at 10 to 40x magnification to examine the water for nematodes (Figure 2).

*Deladenus siricidicola* nematodes can be distinguished from other nematodes because they tend to contort into 'knots' and have a jerky motion in water rather than the swimming motion characteristic of other nematodes. Positive identification should be made by an experienced nematologist.

This method can be used to estimate the distribution of the parasite nematode. The chip detection method can be supplemented during summer by the direct examination of *Sirex* Wasps.

Sample billets of infested trees from a wide area are caged and the adult *Sirex* emergents collected and examined for the presence of the nematode. These examinations involve the severing of the abdomen from the thorax, squeezing the abdominal contents onto a microscope slide, and examining them at 10 to 40x magnification.

### Further information

For information regarding the supply of the parasitic nematodes and on the equipment or techniques described in this report, please contact:

Keith Turnbull Research Institute,  
Land Protection Division,  
Department of Conservation, Forests and  
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P.O. Box 48, Frankston 3199.  
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