The management of Sirex noctilio in South Africa: Standard operating procedures for monitoring and biological control operations

Philip Croft, Brett Hurley and Marcel Verleur

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## Key Findings

- Monitoring the movement of *S. noctilio* in SA has facilitated the immediate release of biological control organism enhancing control measures.
- Standard Operating Procedures have produced consistent inoculation results across SA.
- Monitoring *S. noctilio* population, tree mortality, and parasitism are key to successful management of this pest.
The management of *Sirex noctilio* in South Africa: Standard operating procedures for monitoring and biological control operations

*Philip Croft*¹, *Brett Hurley*² and *Marcel Verleur*³

¹ Institute for Commercial Forestry Research (ICFR), P O Box 100281, Scottsville, 3209, Pietermaritzburg, SOUTH AFRICA
Email: philip.croft@icfr.ukzn.ac.za Telephone: +27 33 386 2314, Fax: +27 33 386 8905, www.icfr.ukzn.ac.za
2 Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria
3 Sappi Forests, P O Box 13124, Cascades, 3202

**Summary**

*Sirex noctilio* is a woodwasp currently threatening commercial pine plantations in South Africa. It was first detected in South Africa in the Western Cape in 1994 on *Pinus radiata*. By 2002, *Sirex* had spread along the Southern Cape and into the Eastern Cape moving onto *Pinus patula*. From the Eastern Cape it moved into KwaZulu-Natal where the population exploded with high tree mortality during 2004. Currently *S. noctilio* has moved throughout the country with a handful of areas where it still remains undetected.

In 2002, the South African *Sirex* Control Programme was formed, and today exists as a collaborative initiative of the South African Forestry Industry, in partnership with the South African National Government Department of Agriculture, Forestry and Fisheries, and is managed through Forestry South Africa, an association representing all timbers growers in the country. Its objective is to develop and implement a strategy to manage *Sirex*, by reducing the wasp population to an acceptable level thereby reducing the impact and risk to South African commercial pine plantations.

The Programme’s strategic approach to manage the risk includes Biological Control (research and commercial initiatives), Monitoring, Awareness and Research to develop a knowledge base of *S. noctilio*. This report provides information on the standard operating procedures currently being used by the Programme for the Monitoring and Biological Control aspects.

**Keywords:**
- National monitoring
- Emergence cages
- SASCP
- *Ibalia leucospoides*
- Inoculation procedure

**Introduction**

*Sirex noctilio* was first reported in South Africa at a timber yard in Port Elizabeth in 1962. However, the wasp did not become established in pine plantations in the area. In April 1994, *S. noctilio* was recorded at Tokai, Cape Town in *Pinus radiata* plantations. Its initial spread was in a 90-km arc through pine plantations of this region. *Sirex noctilio* infestation levels in the Western Cape province of South Africa have generally been low. The only exception was an infestation in George in 2002, where tree mortality was around 10% in 100 ha of 12 to 13-year-old *P. radiata*. These trees were overstocked and the infestation subsided during the course of the next year.

In 2002, *S. noctilio* was detected in the Eastern Cape and KwaZulu-Natal provinces. A variety of *Pinus* species were infested, but mostly *P. radiata* in the Western Cape and
P. patula in the Eastern Cape and KwaZulu-Natal. Infestation levels in the north eastern Cape and KwaZulu-Natal were substantially higher than those reported in the Eastern Cape, with a number of compartments having over 10% annual tree mortality, and some higher than 35% cumulative. In 2005, it was estimated that approximately 35 000 ha of pine in the Eastern Cape and KwaZulu-Natal were infested to a mean level of 6%, with a total estimated value of damage being R300 million per annum (2005).

In 2005, S. noctilio was found around Greytown in central KwaZulu-Natal. During 2007, S. noctilio females were found in panel traps at Vryheid and Ngome in Northern KwaZulu-Natal and by 2008, as far north as Commandale on the border between KwaZulu Natal and Mpumalanga. By 2011 S. noctilio had moved throughout the entire country but remains undetected on a few localised areas.

In 2002, in response to this threat, the South African Sirex Control Programme was formed, and today exists as a collaborative initiative of the South African Forestry Industry, in partnership with the South African National Government Department of Agriculture, Forestry and Fisheries, and is managed through Forestry South Africa, an association representing all timber growers in the country. Its objective is to develop and implement a strategy to manage S. noctilio, by reducing the wasp population to an acceptable level thereby reducing the impact and risk to South African commercial pine plantations. The Programme is managed through a Steering Committee with representation from the Industry and Government as well research partners; the Institute for Commercial Forestry Research (ICFR) in Pietermaritzburg, and the Forestry and Agriculture Biotechnology Institute (FABI) based at the University of Pretoria. Programme activities are directed through an Operational Committee with company representation, and implemented by the coordinator with support from company individuals and contractors.

The Programme’s strategic approach to managing the risk includes:

- Biological Control (research and commercial initiatives)
- Monitoring
- Awareness
- Research to develop a knowledge base of S. Noctilio

This report provides information on the standard operating procedures currently being used by the Programme for the Monitoring and Biological Control aspects.

The most successful biological control agent of S. noctilio is the nematode Deladenus [previously Beddingia] siricidicola. In 1962, D. siricidicola was discovered in New Zealand’s North Island.

Seven species of Beddingia have been found, parasitising some 31 Siricid species. Only Deladenus siricidicola was found suitable for controlling S. noctilio. Deladenus siricidicola has a bi-cyclic life cycle and the individuals in the different phases of the life cycle are morphologically distinct.

- The one phase is free living and feeds exclusively on the fungus Amylostereum areolatum. The emerging juvenile nematodes start the fungus feeding phase, breed up in large numbers and spread throughout the trees. The fungus feeding phase can live in the tree for an indefinite period as long as the fungus is present.

- The other phase is parasitic and feeds on the body fluids of S. noctilio larvae. When juvenile mycetophagous (fungus feeding) nematodes reach the microenvironment around S. noctilio larvae they are stimulated by the high CO2 and low pH, resulting in the development of parasitic adults, instead of mycetophagous adults. After mating, the female nematode bores through the S. noctilio larval cuticle and enters the body cavity of the larva. Here she sheds her cuticle, which leaves the body covered with microvilli, which assists with absorbing nutrients from its host. The female parasitic nematodes later produce juveniles, which invade the gonads of S. noctilio during pupation and penetrate the eggs rendering them sterile. Emerging female wasps oviposit these eggs into selected trees, where the juvenile D. siricidicola emerge from the eggs as fungus feeders (non parasitic form). These nematodes migrate into the trees following the A. areolatum and will search for S. noctilio larvae.

This bi-cyclic life cycle has been exploited to produce one of the most efficient biological control agents against S. noctilio. Nematodes in the mycetophagous phase can be reared in large quantities on artificial cultures of A. areolatum. These nematodes are then inoculated into S. noctilio infested trees where they eventually parasitize the S. noctilio larvae. The emerging parasitized S. noctilio females will then naturally spread the infection throughout the S. noctilio population. The use of D. siricidicola is now recognised as the primary biological control agent against S. noctilio infestations. Once introduced, most of its dispersal is by female S. noctilio with occasional man made re-introductions if required. D. siricidicola has been introduced throughout the southern hemisphere wherever S. noctilio infestations occur.

**Ibalia leucospoides – background**

The parasitoid wasp Ibalia leucospoides has been used internationally to supplement the biological control with D. siricidicola. In South Africa I. leucospoides have been successfully introduced into the Western Cape and KwaZulu Natal, and more recently into Mpumalanga and Limpopo. They have a body length of 16–17 millimetres with black legs (Figure 1). The female abdomen is somewhat elongated, while in males it is pyriform.
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Monitoring Sirex

Determining the presence of Sirex

There are many reasons why trees die, and tree diseases and pests, as well as abiotic factors, can produce similar symptoms in trees. Therefore, a combination of characteristics is required to positively identify S. noctilio infestations. It is vital to understand how S. noctilio kills trees if we are to control S. noctilio population levels.

*Sirex noctilio* is capable of killing trees by injecting a phytotoxic mucus secretion and a fungus during oviposition. Although *S. noctilio* is able to weaken and kill healthy pine trees, they are particularly attracted to suppressed trees in un-thinned or over-aged stands and drought-stressed trees. The susceptible age for initial attack in a suppressed tree is from 10 years upwards in high stocking. When the *S. noctilio* population increases, healthy trees are also affected.

*Sirex noctilio* females oviposit between 30 to 450 eggs depending on the size of the female. *Sirex noctilio* females drill into the sapwood of selected host trees. If the tree is found suitable, then eggs are deposited with a second tunnel drilled in the same location depositing a mucus secretion and spores of the fungus *Amylostereum areolatum*. The toxic mucus prevents the translocation of sugars from the foliage to the site of infection and prevents the formation of polyphenols, which act as anti-fungal agents. The developing mycelium destroys the vascular system of the tree and causes the tree to dry out. The tree rapidly wilts and the crown discolours.

*Sirex noctilio* eggs hatch after about 10-12 days when the fungus in the area has become established. The larvae remain at this site during the first and second instar. From the third instar onwards larvae tunnel through the wood while feeding on *A. areolatum*. The average number of instars is 10–12, but under adverse circumstance, even younger instar larvae are able to pupate resulting in smaller adults. Pupation lasts 16–21 days. *Sirex noctilio* generally completes its life cycle in 10–13 months, but it can take up to two years in colder climates. The size of the adults is determined by the conditions within the tree, which determines fungal growth development. Adults do not feed and only live for a few days. The flight period is late summer / autumn in the European temperate climate zone and late autumn in the Mediterranean areas. In Australia, adult emergence occurs from January to May (late summer / autumn). In the winter rainfall region of South Africa the *S. noctilio* flight period is from December to April (mid summer – autumn). The flight period in the summer rainfall region is from the end of October to January (early to mid summer); usually by the end of November more than 80% of the *S. noctilio* adults have emerged.

Several key indicators confirm the presence of *S. noctilio* in plantations (Figures 2 and 3):

- Round emergence holes are visible where the wasps have emerged from the tree. These holes continue into the wood when the bark is removed, unlike the holes of the pine weevil, *Pissodes nemorensis*, which are also circular but only go through the bark.
- The crowns of *S. noctilio* infested trees start to wilt and turn yellow. They will later turn red and the needles will start to drop off. In the summer rainfall areas, the crown starts to wilt in February and continues to wilt through to May. The needles start to drop off in July.
- The wilting and discoloring of the crown occurs throughout the entire crown as opposed to fungal infections where the discoloration starts from the tips of branches spreading towards the trunk.
- During the flight period, female wasps can be seen either actively ovipositing in the tree or dead, but still with the ovipositor stuck in the tree.
• Resin droplets occur on the stem where the ovipositor penetrated the tree to lay the eggs.
• When dead/dying trees are felled and cross cut, *S. noctilio* tunnels can be identified as round tunnels with tightly packed fine sawdust. The tunnels will contain larvae in them from February to November in the summer rainfall regions. The larvae are creamy white with a black posterior spike.

Once it has been established that *S. noctilio* is present at a location, the condition of the trees can give an indication of how long it has been present at that location (assuming that all *Sirex*-attacked trees in that area are detected):
• 1st season: Trees with brown or bare crowns and no exit holes present.
• 2nd season: Dead trees with bare crowns and exit holes with the timber still hard when struck with the blunt end of a hatchet.
• 3rd season: Trees as in (2) with timber soft (dents) when struck with the blunt end of a hatchet.
• 4th season and older: Trees as in (3) which have fallen down due to wind and showing excessive dry rot.

![Diagram](image)

**Figure 2:** Protocol for confirming the presence of *Sirex noctilio*. 

**PRESENCE OF THE SIREX WOOD WASP CONFIRMED**
Monitoring using a trap network

Monitoring initiatives by the South African Sirex Control Programme, using an extensive network of traps set up across the country, have been effective in facilitating the early detection and subsequent intervention for S. noctilio outbreaks in South Africa. Traps are used ahead of the S. noctilio front, to detect the presence of S. noctilio, and provide an early warning system of new outbreaks, facilitating the early intervention of management initiatives, including biological control.

Panel traps and lures

The Panel Trap™ consists of 4 main parts: 1) Hood, 2) Main body, 3) Collecting funnel, and 4) Plastic collecting bucket. Parts 1, 2 and 3 are built either from black coryx or wood. A wire is attached at the top to attach the trap to a branch or pole, and four retainers connect the collecting cup to the funnel. These traps are highly effective for capturing Sirex woodwasps.

In combination with the Panel Trap, a lure is used to attract the S. noctilio woodwasp and is known as the Sirex PheroLure™. The Sirex PheroLure™ is a combination of six plant volatiles. The lure should remain active in the trap during the flight season. Lures are still active when there is visible liquid in the dispenser. To check for lure longevity, or ongoing activity, inspect the lures to see if any liquid remains in the vials or bags. When the lures dry out, replace with a fresh lure. Lures should be stored in the freezer until installation. Hang lures with a cable tie from the center of the trap panel (as per assembly instruction sheet from supplier). Any extra Sirex PheroLures™ should be kept in their individual foil pouches in a fridge in order to prolong their shelf life.

Trap Management

In the summer rainfall regions of South Africa (KwaZulu-Natal, Mpumalanga and Limpopo), traps need to be operational from the last week of October.

The trap servicing is as follows:

1. The trap must be hung from a tree branch or Γ-shaped pole 2.5 m above the ground (3.5 m pole required) The lure should be 2 m above ground level.
2. Each site should have three traps, each approximately 100 m apart.
3. The pole must be outside the compartment and in an area of pine trees older than 12 years. If the trap is hung on a tree, it is preferable to select either an adjacent eucalypt or wattle compartment in preference to pine trees, as the Kairomone lure will be more easily detected.
4. Label the trap bucket / bowl with a marker pen indicating the trap number e.g. 25A or 25B or 25C.
5. The traps may be hung in advance, but the lure must only be attached at the start of the S. noctilio woodwasp flight season.
6. Water should be placed in the collecting bucket. (The bucket must be half full with water).
7. Three small drops of dishwashing liquid are added into the water (slightly soapy), to reduce the surface tension of the water, preventing the insects escaping.
8. The traps must be inspected weekly to remove the insects from the collecting bucket and replace the dishwashing liquid in the bowl.

Figure 3. Sirex noctilio indicators.
9. If the collecting bucket contains many insects then it must be emptied more frequently.
10. Use a strainer to remove excess water from the insects and place the insects / wasps in a plastic bottle containing ethanol (at least 70%), to preserve the insects.
11. The bottle must be labeled as follows:
   i. Trap number
   ii. Date of insect collection
   iii. The label must be plain white A4 paper
   iv. Write with a dark pencil (Only pencil – no markers or pen)
   v. Place the label in the bottle with the ethanol and insects
12. All insects from a site must be placed in separate bottles e.g. Site 25 has trap A, B and C. All insects from trap 25A are placed in one bottle per date of collection.
13. One kairomone lure will last about a month. Each trap will receive two lures; one is put out for the first four weeks of the flight season. The other is kept in a fridge for four weeks then put up on the last week of November for the remaining four weeks of the eight week flight season.
14. The traps are monitored until the end of December (for summer rainfall areas) and after this they must be taken down.
15. The bottles with insects must be stored in a coolroom (out of sunlight to prevent the alcohol from evaporating) and with lids facing up while waiting for collection.
16. Equipment required for managing the traps:
   - Ladder
   - Water (in containers)
   - Pliers / Side cutters
   - Marking pen – to mark the bowl
   - Dishwashing liquid
   - Sieve
   - Bottles for the insects and boxes or crates to hold the bottles
   - Paper for labels
   - Pencils
   - Ethanol (clearly labeled – POISONOUS) (Decant into smaller bottles for easier use)
   - Spare cable ties should a trap fall down
   - Small cable ties to secure the lure to the trap
   - Maps
   - Spreadsheet with contact details and trap numbers
   - Plastic jug (to pour the water from the trap through the strainer into the jug, so that the water can be poured back into the trap bucket)
   - Pen and paper to record missing / stolen / destroyed traps and any other details that are found while inspecting the traps

Disposal of lures

Sirex PheroLure™ that is due for replacement or of no use can be disposed of in the garbage. No special precautions are required as the PheroLure™ is non-toxic.

Monitoring using trap trees

Trap trees can also be used for the early detection of S. noctilio and intervention through bio-control. Trees are artificially stressed with herbicides in a known location to attract S. noctilio females to oviposit. The timing of trap trees is critical as the tree must release the volatiles that attract S. noctilio during the woodwasp flight season.

Trap tree plots for early monitoring purposes are placed at an intensity of one plot of ten trees per 200 ha of pine older than 12 years. Once the presence of S. noctilio is confirmed in an area, the intensity for inoculation purposes is increased to one plot of ten trees per 100 ha of pine older than twelve years. When emergence and dissection data indicate that background levels of nematode parasitism in an area are well established, the intensity of trap tree plots reverts back to monitoring intensity.

In saw timber rotations trap trees can be selected from trees which usually would be taken out during thinning operations. In pulpwood operations trap trees are usually placed in a row of ten adjacent trees, with no selection with regard to form or size.

Trap trees are prepared four to six weeks before the peak emergence of S. noctilio. In the winter rainfall regions trap tree plots should be prepared during the first two weeks of January and in the summer rainfall regions trap tree plots should be prepared during the first two weeks of October each year.

The herbicide Dicamba is used to prepare trap trees in most countries where trap tree plots are deployed. This product is not commercially available in South Africa. Trials conducted in KZN have indicated that a mixture of Dicamba, 24D and MCPA [marketed as Super Lawnweeder and Turfweeder] is the best commercially available product to stress Pines for trap tree plots. However, one must caution that these individual chemicals are all on the FSC hazardous chemicals list and that the brand products are not registered for this specific use. As an alternative, Glyphosate can be used but trials have indicated varied success.

Trap trees are prepared as follows:

- Horizontal cuts are made with a hatchet, 10–15 cm apart around the circumference of the tree at about 1 m height. (Average 4 cuts per tree)
- 2 ml of undiluted Super Lawnweeder is injected into each cut (average 8 ml per tree)

National Monitoring

National monitoring plots are laid out and the trees that are living, dying due to S. noctilio attack, dead from S. noctilio activity and other symptoms are recorded. This information indicates tree mortality at a compartment level and can be combined with other plots to give an indication of tree mortality from farm up to provincial level.

The plots are line transects 75 m long and a minimum of three transects are laid out at every plot location. A minimum of 75 trees must be assessed and if 75 trees are not found in the three line transects then additional transects are laid out until a minimum of 75 trees have been recorded. The plots
are randomly selected in GIS based on certain preconditions such as pine, over the age of 10, and 80% of the plots in high risk areas and 10% of the plots in low risk areas.

One site with three plots is established for every 600 hectares of mature pine and based upon data published by DAFF in 2009 (Forestry Branch, Report on Commercial Timber Resources and Primary Roundwood Processing in South Africa, 2009) which indicated that there are 324,000 hectares of pine between the ages of 10 and 20 years.

Annually the GIS database is updated with the latest company plantation data to ensure the plots remain in areas where fires and harvesting operations have not taken place. The plots will remain in their original location unless harvesting or fires or access has become a problem. When the plots need to move a new location is sought within the GIS program and the new plot is established.

The first and last tree is marked in each transect with yellow paint to indicate the plot location. The first transect and first tree being 11 and the second tree marked 12 until the 75 m mark is reached. The second transect first tree marked 21 and second tree marked 22 continuing until the 75 m mark is reached. Each tree in each transect is numbered so the following years assessment takes place on the same trees in the same plot. This assists when thinning operations have taken place and trees in the plot were removed.

Recording sheets are completed by the contractors and forwarded to the SASCP co-ordinator for data capture and analysis. These results are released annually.

The recording table (Figure 4) and can be completed as an excel spreadsheet and emailed to the co-ordinator for data capture.
### Sirex National Monitoring - Recording Sheet

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**Figure 4.** Monitoring plot recording sheet.
Biological Control

Nematode handling procedures

Supply of nematodes

Currently, the Forestry and Agriculture Biotechnology Institute (FABI) at the University of Pretoria are supplying the South African Sirex Control Programme with nematodes.

Timing of inoculations

The optimum inoculation period for the summer rainfall region of South Africa is considered to be from mid February to April. Prior to March, many of the dying trees are not obvious, while by July the moisture content of dying trees is likely to be too low to ensure success. [Sapwood moisture content at time of inoculation of > 50% is considered preferable]. The winter rainfall period requires inoculations during May to July. In the summer rainfall regions, trap tree plots are inoculated during February while there is still adequate moisture in the trees.

Management of the nematode supply chain

Effective and efficient management of the nematode supply chain is vital to ensuring survival of the nematodes, and results in successful inoculations.

• Nematode survival is paramount. The process must cater for both fail-safe and practical-operational flask-transport-tree supply chains. This applies to all D. siricidicola deliveries that need to be in place before the 1st inoculations are due.

• Checks have to be conducted at each step of the nematode supply chain, from the laboratory to the tree.

Nematode care and handling

• Nematodes are transported in sealed plastic bags containing 1 million nematodes per 10 ml, generally with 1 million or 5 million per bag. These should be packed in insulated boxes with freezer blocks and kept between 5 and 10 °C at all times. When stored in a refrigerator, a maximum / minimum thermometer should be included to ensure the acceptable temperature range is maintained. The optimum storage temperature is 5 °C, but must not reach 0 °C. At higher temperatures, the nematodes use more oxygen thus increasing the probability of nematode mortality.

• Nematodes should be used as soon as possible and certainly within ten days after they have been flushed from the production cultures. Under optimum conditions they should be able to be kept up to five days after receipt.

• Samples of nematodes should be checked under a microscope prior to mixing, to ensure they are alive.

Quality control of nematodes

A central body or co-ordinator controls the distribution of nematodes to the various companies and arranges quality checks as to the virulence, survival and numbers of nematodes per packet.

Protocol for testing nematode quality

1. Immediately upon receipt, measure the temperature of the nematodes (this should be between 5 °C and 10 °C).
2. Count the number of sachets received and checks for broken sachets.
3. Randomly remove approximately 10% of the bags sent.
4. Keep these bags in a cool container or in the fridge (5 °C) while working with them.
5. For each bag (one bag at a time):
   i. Fill a beaker with about 400 ml of water at 5 °C for each sachet selected.
   ii. Empty the contents of one sachet into the beaker. Flush out the remaining nematodes / contents with clean water at 5 °C.
   iii. Top up the beaker with water at 5 °C to 500 ml.
   iv. Continuously blow through a pipette or straw or use a fish tank pump, to keep the nematodes in suspension while drawing out 1 ml of the solution.
   v. The 1 ml is placed in a sample bottle containing 19 ml of water at 5 °C. (Total volume is 20 ml). Label the sample bottle.
   vi. Step iv and v are repeated five times (five sample bottles).
   vii. Step i to step vi are repeated for each sachet sampled.

Counting Process

   viii. Shake the sample bottle to suspend the nematodes and draw 1 ml from the sample bottle and place onto a Hawksley slide or petri dish with a grid.
   ix. The Petri dish should be allowed to stand to allow the nematodes to settle to the bottom.
   x. Allow the nematodes to warm up to room temperature (seen moving around) before commencing counting. The period for this will depend on how cold the nematodes were on arrival, but will normally require 20-30 minutes.
   xi. Count all the nematodes in all the grids of the Hawksley slide or petri dish.
   xii. Multiply the number of nematodes counted by 10,000. This will give you the number of nematodes in the sachet (5,000,000 nematodes per sachet should give a count of 500 nematodes per slide).
   xiii. The sample bottles should be kept at 5 °C for future reference.
   xiv. The solution from the sample sachets are then poured into bottles and despatched to the field.
6. Keep the nematodes in a fridge at 5 °C until they are collected by the inoculation team.
Precautionary measures

1. Ensure the pipette is calibrated accurately. This can be done by pipetting 0.5 ml or 1 ml water into an 0.5 ml Eppendorf or 1 ml Cryo-vial.
2. Only juvenile nematodes should be counted. Mature or adult nematodes are not to be counted.
3. The assessment of a nematode as to whether it is an adult or juvenile can be subjective, and this will be discussed at the annual training session.
4. All laboratory technicians involved in counting nematodes at laboratories will be trained annually by FABI staff to ensure the correct assessment of adult and juvenile nematodes.
5. Annual refresher training will be carried out prior to the new inoculation season supply of nematodes being despatched from FABI.

Dispatch of nematodes to inoculation teams

Nematodes must be transported at 5 °C and a fridge is required for this purpose. The sachets can be laid flat separated with corrugated cardboard to allow air to flow between the sachets.

Preparing nematodes for inoculation

Nematodes must be mixed with Stockosorb®, a potassium polyacrylate powder, which forms a gel, to hold them in the inoculation hole until they move into the tree tracheids. A mixture of 500 ml of nematodes in gel mix is sufficient to inoculate 10–12 standing pulpwood trees.

Protocol for preparing nematodes for inoculation

- Keep nematodes at 5 – 10 °C, preferably 5 °C.
- Only prepare enough nematode mixture for one day’s inoculations. The nematode mixture must be used within 36 hours after preparation, preferably the same day.
  i. Pour 500 ml refrigerated (5 °C) tap water into mixing bowl per million nematodes in the bag. Note: 1 million nematodes = 500 ml, 5 million nematodes = 2 500 ml
  ii. Empty one sachet of nematodes into the water and stir gently to evenly distribute nematodes.

iii. Add Stockosorb® while stirring. Stir for 2-3 minutes. The mixture will become a thick gel. Note: 5 g Stockosorb® powder = 1 million nematodes, 25 g Stockosorb® powder = 5 million nematodes

iv. Pour into 500 ml tomato sauce dispensing bottles and close with the spout cap.

v. Store at 5 °C and transport to field (e.g. use cooler box with freezer blocks / bags and thermometer to ensure temperature is about 5 °C; remove or add freezer blocks to obtain correct temperature; ensure that plastic bottles with nematodes are not in direct contact with freezer blocks, i.e. cover freezer blocks with cloth etc.).

Inoculation procedures

- Only currently Sirex-infested trees are inoculated. If exit holes are present and the timber is very dry, there are most likely no S. noctilio larvae in that tree. Occasionally there are two life cycles in a tree and the wetter portion without exit holes may need inoculation.
- Each tree should be distinctively marked or numbered for later identification.
- When inoculating in the plantation, the ambient temperature should not exceed 20 °C under the tree canopy.
- Ideally trees should be inoculated in the early morning and late afternoon.
- Avoid inoculating on rainy days, as the gel may wash out of the inoculation holes.

Inoculation tools

- Inoculation hammers are critical in the process and must be in good condition. This especially applies to the rubber end of the hammer.
- Inoculation punches need to be kept sharp and round at all times using a chainsaw file. Punches must cut cleanly through the tracheids so that nematodes can readily move from the gel into the wood. Spare punches are necessary should a punch become blunt or damaged. Lubrication of the inside of the punch (especially overnight) maintains cutting performance and ejection of wood cores.
- A local manufacturer in Pietermaritzburg, KwaZulu-Natal manufactures the hammers and sharpens the punches.
Checklist for inoculations with nematodes

Table 1. Checklist for nematode inoculations

<table>
<thead>
<tr>
<th>Office or suitable preparation area inside:</th>
<th>Check</th>
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</thead>
<tbody>
<tr>
<td>1 Worksheet titled “Protocol to Inoculate Trees ...”</td>
<td>Check</td>
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<tr>
<td>2 Nematode sachet (provided by FABI)</td>
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<tr>
<td>3 Sachet / container of polyacrylamide gel (provided by FABI)</td>
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<td>4 500 ml measuring jug</td>
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<tr>
<td>5 Mixing bowl (1-2 litres in size) and plastic spoon</td>
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<tr>
<td>6 500 ml tomato sauce bottles</td>
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<td>7 Cooler box</td>
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<td>8 Freezer blocks and cloth or something to cover them</td>
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<td>9 Thermometer</td>
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<th>In Field:</th>
<th>Check</th>
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<tbody>
<tr>
<td>1 Worksheet titled “Nematode Inoculations: Record Sheet”</td>
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<tr>
<td>2 Chainsaw safety equipment</td>
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<tr>
<td>3 Inoculation hammers (at least two per team). Ensure that cutting edge is sharp</td>
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<tr>
<td>4 Spare punches for hammers and Allen key</td>
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<tr>
<td>5 Timber sealant and brush to apply</td>
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<tr>
<td>6 Dispensers e.g. sauce bottles</td>
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<tr>
<td>7 Knife or scissors to cut bag with nematode-gel mixture</td>
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<tr>
<td>8 GPS, if inoculating sites where coordinates unknown</td>
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</table>

Inoculating sawtimber

- The tree must be felled to give access to the regions where the S. noctilio female wasp laid her eggs. The trees should be marked before the felling team arrives and the trees should be counted to facilitate the record keeping system.

- The trees for inoculation should be felled and the branches trimmed to facilitate inoculation, but keeping all the bark intact.

- Once felled, the cut ends of the trees should be slightly raised off the ground and the ends sealed with sealant (Acrylic roof sealant or PVA paint) to prevent rotting, moisture loss and blue-stain fungus spreading through the tree.

- Tree inoculation is usually carried out by teams of two or three people. One person punches holes while the other(s) follow doing the inoculating.

- It is important that the hole punching process along the whole tree is completed before the gel is applied. This will ensure that the gel does not bounce out of the hole(s) while the holes are being prepared.

- An average of 15 - 25 trees can be cut and inoculated per person per day, depending on travelling time, tree size and terrain.

- Inoculation holes approximately 10 mm deep are made using the rebound hammer. Hole spacing should be as follows:
  - For Log diameters >15 cm – 2 rows of staggered holes 30 cm apart along the log in about the 10 to 12 o’clock position.
  - For Log diameters < 15 cm – 1 row of holes 30 cm apart along the top of the log down to about 5 cm diameter.
  - There is no need to inoculate in the deep fissured bark at the butt of the tree, as S. noctilio is less likely to inhabit this part of the tree. Should S. noctilio resin droplets be visible in this lower portion of the tree, then it should be inoculated. Use a spade or hatchet to remove the thick bark, (leaving some bark on the tree) and then proceed to make the inoculation holes (Figure 5).
Diagram showing correct hole placement

Figure 5. Diagram indicating correct hole placement in a felled sawtimber log.

- The tip of the gel dispenser is inserted into the base of the inoculation hole and withdrawn as the gel is squeezed into it. Once the hole is filled, the gel is compacted by pressing with the finger or thumb. This compaction is very important as it ensures contact between the gel and the inside of the hole so that the nematodes can move out into the wood easily.
- The un-inoculated section of the tree above the B2 is termed M or Middle and the top is referred to as T.

**Inoculating pulpwood with chainsaw and ladder height restrictions (Mondi Procedure)**

- The trees are left standing.
- The person with the rebound hammer punches a line of holes 30 cm apart from about knee high to as high as he can reach on four sides of the tree. This is the B1 section of the tree.
- A 3 m pole ladder is then placed against the tree and fastened to the tree.
- The person with the rebound hammer punches holes 30 cm apart, from where the lower row of holes ended to as high as he can reach, on two sides of the tree by going no higher than 2 m up the ladder. This is the B2 section of the tree.
- The person with the tomato sauce dispenser inserts the tip of the gel dispenser into the base of each inoculation hole, squeezes the gel into the hole while drawing the tip out of the hole as it fills. Once the hole is filled, the gel is compacted by pressing with finger or thumb. This compaction is very important as it ensures intimate contact between the flummery and the inside of the hole so that the nematodes can move out into the wood easily.
- The un-inoculated section above the B2 is called M or Middle log.

**Exceptions to the above**

- Where the diameter of the tree is small and the chance of the ladder with the weight of the man against the tree my cause the tree to fall over, then such trees will be inoculated on two sides only, and without the use of a ladder.
• When trees are 18 years and older the SASCP standard is to fell and inoculate the felled tree. The specific instruction on Mondi property is that these trees will be inoculated according to the Pulpwood procedure above, and not felled.

**Implications of the specific Mondi procedure**

• The SASCP SOP indicates that 50 holes are placed in each tree and filled with the nematode and gel mixture. Due to the height restriction only 40 holes per tree will be made and filled with the nematode and gel mixture.

• The reduction to 40 holes per tree implies that the number of trees inoculated will increase, but only the lower portions of the tree (where fewer wasps emerge) will be inoculated. The SOP indicates that 100 000 nematodes are to be inoculated into a tree, while this Mondi procedure allows for 80 000 nematodes per tree.

• The selection of B2 logs (logs from the portion of the tree where the ladder is used for inoculating) will be more difficult as there is less inoculated timber available for the sampling process. This could compromise the parasitism results.

• While the majority of wasps emerge from the higher regions of the tree, these are usually male wasps, but the lower areas (now unreachable) may have female wasps emerging which may, under this new procedure, not be parasitised.

**Recording the inoculation process**

A Nematode Inoculation Record Sheet should be completed once the compartment has been inoculated. An example is provided.

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**Compartment Inoculation Record**

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<th>Inoculations done by:</th>
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<th>Date:</th>
<th>Time:</th>
<th>Company:</th>
<th>Plantation:</th>
<th>Compartment:</th>
<th>GPS:</th>
<th>Species:</th>
<th>Age:</th>
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<tr>
<td>Trap Tree Plot (T) or naturally infested (N) trees?</td>
<td>How many trees inoculated?</td>
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<th>Inoculations done by:</th>
<th>T °C</th>
<th>Date:</th>
<th>Time:</th>
<th>Company:</th>
<th>Plantation:</th>
<th>Compartment:</th>
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Emergence cages (drums)

Emergence cages or drums are set up at central locations in George, Howick and Vryheid to collect wasps for dissection, to determine the effectiveness of the biological control programme. Currently, the dissections are carried out at FABI in Pretoria.

Selection of logs for the emergence cages

- Cut logs 80 cm in length from selected inoculated and un-inoculated sample trees.
- Select logs that definitely have *S. noctilio* larvae. (Check the cross cut surface for evidence of tunnels).
- Ensure labelling and numbering details are carefully recorded.
- Place these logs in drums with nets secured over the opening.

Wasp collections

- Inspect the emergence drums daily from the last two weeks in October (KwaZulu-Natal) until all *S. noctilio* emergences cease. For the winter rainfall region, this is from November to April.
- Capture all emerging wasps daily, and place them in sample bottles. The wasps from each cage must be placed in a sample bottle allocated to that cage with the cage number and date recorded on the lid of the bottle.
- Ensure the number on the sample bottle corresponds with the drum numbers.
- Record the number of wasps caught per drum daily.

Wasp sampling

- Male wasps are collected daily, from Tuesday through the week until Monday morning, when they are counted and checked against the records. Then three wasps (3 per 100 and 6 per 200 wasps caught in a particular cage) that are alive are selected and placed in a bottle which is marked with the cage number and packed for transport to FABI for dissection. The remaining wasps are killed once all the wasps have been checked and the records balance with the number of wasps counted. If three wasps are collected but some of them are dead, then some dead ones must be sent to FABI only if there are insufficient living wasps.
- Female wasps are collected from Tuesday morning through to Monday morning. They are counted and the count is verified against the records. Then 10 living female wasps are selected and placed in a bottle marked with the cage number and packed for transport to FABI. If there are more than 10 then the additional females are killed.
- Both male and female wasps that are selected for dissection must be representative of the wasp size caught in the cage for that week.

Transferring *S. noctilio* wasps

*Sirex noctilio* wasps may need to be transported for several reasons:

1. To be dissected to determine if the *S. noctilio* wasps were parasitised by the nematodes, *D. siricidicola*;
2. To collect the *D. siricidicola* from *S. noctilio* for further breeding;
3. To verify the genotype or strain of the *S. noctilio* and thus potentially determine the origin of the wasp (where needed).

The length of time spent in transport will affect the condition of the wasps, and if live nematodes are to be extracted from the wasp for breeding purposes, the handling of the wasps and speedy dispatch is critical. Dispatch should be done at least once per week to prevent the wasps and the nematodes within from dying.

The following considerations should be made:

- Store the wasps in bottles with holes in the lid for air circulation. Use plastic bottles, as glass bottles may break in transit. Ensure that the wasps are securely sealed in the plastic bottles and that the bottles are securely packed in a box. It is crucial to avoid wasps escaping in transit.
- Do not place more than 20 wasps per 100 ml bottle as they attack and mutilate each other.
- Label each bottle with the following information:
  - Cage number from which they were collected
  - Date of wasp collection
  - If the wasps are to be kept for a few days before transport, keep the bottles in a fridge at 5 °C.
- When ready to be dispatched, the bottles should be placed in a cooler box with two ice bricks to keep the wasps cool but not frozen. Separate the bottles and the ice bricks with cotton wool.
- Notify the recipient of the wasps at dispatch. Communication between the sender and the recipient is very important.

*Ibalia leucospoides*

Handling and transportation

- Handle with care, and minimise or avoid handling them with the hands.
- Catch the wasp by placing the bottle over the wasp and allowing it to move into the bottle or container.
- Bottles must have small holes or gauze in the lid to allow airflow. Only use plastic bottles, as glass bottles may break in transit.
- Preferably add shreds of dry tissue or toilet paper inside the container for the wasps to hold on to before and during transport. Important not to have something heavy in the bottle that could squash them.
- Feed the wasps with honey-water. Mix one drop of honey in a teaspoon of water (or a teaspoon of honey in half a cup of water). Apply this mixture to the inside of the gauze on the lid. Alternatively, make cotton wool damp in the mixture, but not drenched, and stick this on the inside of
the lid. Leave in a shady, cool area for half a day or more to feed.

- NB. Remove wet cotton wool from container before transport.
- Store in fridge (5-10 °C) until wasps are dispatched.
- Send in cooler boxes (polystyrene) with two ice blocks per box.
- As with transporting S. noctilio wasps, notify the recipient of the wasps of their dispatch date and time and their expected arrival day and time.
- Check that the parcel is delivered. Communication between the sender and the recipient is very important.

**Release into the plantation**

- The bottles must be kept cool (5-10 °C) during transport.
- Ensure there is nothing heavy in the bottle that could damage the insects should the bottle move or jolt in transport.
- Find a plantation area and tree that is S. noctilio infested.
- Open the bottle and allow the wasps to walk onto a piece of cardboard held against the stem of the S. noctilio infested tree.
- The wasps will gradually fly off or climb onto the tree stem.
- Record the location for future capture and release operations.

**Considerations**

- If *I. leucospoides* are transported from different geographical locations around the country (e.g. Western Cape to KwaZulu-Natal), the timing of the S. noctilio egg laying and the release of *Ibalia* should be considered. To ensure the S. noctilio larvae are still available as a food source to *I. leucospoides*, *I. leucospoides* should not be released more than three weeks after the S. noctilio flight period has ceased.
- If the timing is such that the S. noctilio eggs and first instar larvae are no longer available, the *I. leucospoides* may be used for breeding purposes.
- If the *I. leucospoides* are to be used for breeding purposes, they should be sent to FABI with the necessary notification period to ensure they have Sirex-infested logs with S. noctilio larvae at the right instars development.

In summer rainfall areas *I. leucospoides* can be released from November to January to coincide with the S. noctilio emergence period. In winter rainfall areas the *I. leucospoides* should be released from December to April following the emergence period of S. noctilio.