12413.

Training on biological control of *Sirex noctilio* with the use of parasitoids

Participant: G.D. Tribe
Venue: Curitiba, Brazil
Duration: 4 - 9 November 1996

Contents: **Page**

Report on the course "Training on biological control of *Sirex noctilio* with the use of parasitoids" held in Curitiba, Brazil from 4 - 9 November 1996

Summary

Attendance of this course allowed both the technical expertise to be learnt in rearing the hymenopterous parasitoid species of *Sirex* as well as valuable contacts to be made with forest entomologists in Latin America. There is also a possibility that these parasitoids may be purchased at a cheaper rate than that demanded by the Australians at Aus.$ 100 per female parasitoid.

Plant Protection Research Institute was to have introduced only two of the parasitoid species viz. *Megarhyssa nortoni* and *Ibalia leucospoides*, but the consensus from the experts is that *Rhyssa persuasoria* should also be imported. *Rhyssa persuasoria* parasitises late instar larvae of *Sirex* in smaller diameter logs and branches and is mostly found in the crown region. In this way it complements *Megarhyssa nortoni* which confines itself to the thicker lower stump. The parasitoids on their own have the ability to achieve an 80 % parasitism rate of *Sirex*.

It was pointed out by Dr. Madden that the low percentage parasitism achieved in South Africa with the nematode *Deladenus siricidicola* and our determination that the nematode failed to disperse within the logs, could be the result of blunt inoculation hammers. This has now been verified.

Although it was recommended that the parasitoids be introduced within the first six years after *Sirex* is discovered in a country, the population level of *Sirex* is presently so low in South Africa due to our remedial actions that establishment of the parasitoids will be much more difficult than in Brazil.

When compared with the Brazilians, forest protection in South Africa is vastly under-funded yet the expertise in South Africa is of a very high standard.

Recommendations:

1. That *Rhyssa persuasoria* should also be imported.
2. That negotiations to purchase the parasitoids be made directly and not through third parties.
3. That less expensive parasitoids could be purchased from the South Americans (which would also offset their initial costs).
4. That funding of the *Sirex* project will also have to be increased substantially.

General

Plant Protection Research Institute has been contracted to import and establish the two most important hymenopterous parasitoid species (*Ibalia leucospoides* and *Megarhyssa nortoni*) of *Sirex noctilio*. Because *S. noctilio* is presently confined to the Western Cape in a 90 km arc around Cape Town, this importation will be done through the Rosebank station.

The above course was sponsored by EMBRAPA (Brazil), the USDA Forestry Service, the International Institute for Biological Control (UK) and the International Union of Forest Research Organizations. Its purpose was to train researchers in all countries recently invaded by *S. noctilio* in the theory and practice of importing the hymenopterous parasitoid species as additional support for the biological control program begun with the importation of the parasitic nematode *Deladenus siricidicola*. Latin American countries surrounding Brazil were invited to send candidates to the course, with an invitation also for two participants from South Africa. Mr. Waldo Hinze, Senior Manager of Research and Planning, representing SAFCOL which has already contributed much to the neutralising of *Sirex* in South Africa, was one candidate. Dr. G. Tribe of P.P.R.I. was funded by the Forest Owners Association to learn the technical aspects involved in the importation of these parasitoids.

The key speaker was Dr. John Madden of the University of Tasmania who was involved in the control of *Sirex* from the outset once it had reached Australia. Much of the theory was given by Dr. Sean T. Murphy (IIBC) based at Silwood Park in the U.K. His introductory talk listed the recent successes in biological control in forests throughout the world and several of these were P.P.R.I. successes such as that of *Trachymela tincticollis* with *Enoggera reticulata, Phoracantha* with *Avetianella longoi*, and *Cinara cronartii* with *Pauesia cinaravora*.

Other key speakers included Dr. Edson Tade Lede, head of Embrapa Florestas protection services and ultimately responsible for the success of the biological control of *Sirex*. He was ably assisted by Susete Penteado who lectured on percentage parasitism, the distribution of *Sirex* within logs, and the optimum temperature and RH at which to store hymenopterous parasitoids.

Questions were answered at the end of each talk and many general discussions were held. Although representing many different countries, the delegates all responded very openly and amicably throughout the entire course and much of the credit for this goes to Embrapa and their chief organiser of the course, Erich Schaitza.

A two day field trip was organised to Lages which is 400 km south of Curitiba, an area which lies at the heart of the pine growing region. Here we were shown stressed trees (due to lack of thinning) heavily attacked by *Sirex*, trap trees etc. An instant impression was that there were many more *Sirex* larvae per cubic metre in their *P. taeda* logs than would be found in *P. radiata* in South Africa. In addition the trees attacked were much younger (10 - 15 yrs) and the larvae had bored deep into the heartwood. The general impression was that their larvae were consistently much larger than those found in South Africa. The reason for these differences may be the warmer sub-tropical climate with rainfall throughout the year which may be conducive to both the faster growth of the *Sirex* larvae and the slower drying out of the attacked trees.

Introduction:

Sirex noctilio was discovered in southern Brazil in 1988 whence it had dispersed after first appearing in Uruguay in 1980. Biological control was begun in 1990 with the introduction of the parasitic nematode *Deladenus siricidicola* from New Zealand. Brazil has two million hectares of pines (of which 1 million ha. occur in the south) and 90 % of these are *Pinus taeda*. Brazil was fortunate enough to have the egg and early larval parasitoid *Ibalia leucospoides* arrive with the *Sirex* wasp. Although *D. siricidicola* is regarded as the key biological control agent in Australasia with up to 90% parasitism in certain regions, the importation of additional biological control agents in the form of hymenopterous parasitoids was considered necessary to prevent possible irregular upsurges in *Sirex* numbers. This is because the hymenopterous parasitoids are most effective when their *Sirex* host population is at low levels. In Tasmania, before the introduction of *D. siricidicola*, the seven parasitoid species were able on their own to account for up to 80% of the *Sirex* population and are therefore an integral part of the biological control programme.

The two parasitoid species to be imported by Brazil are *Megarhyssa nortoni* and *Rhyssa persuasoria*. The two main speakers at the training course were Dr. John Madden of the University of Tasmania who was involved from the earliest days in the introduction of biological control agents of *Sirex* into Australia, and Dr. Sean Murphy of the International Institute of Biological Control in England. The Brazilians therefore organised this course and invited all countries involved in the biological control of *Sirex* to attend. There were 32 participants including the speakers on the course, representing Brazil (16), Chile (6), Argentina (4), Paraguay (1), Uruguay (1) and South Africa (2).

The course not only allowed one the opportunity to learn the intricacies of rearing both *Sirex* and their parasitoids in the laboratory but also to make contact with forest protection researchers of Latin America. This will hopefully be to our advantage in the future due to the large number of forestry pests which have appeared in South American countries and which may at some stage find their way to South Africa.

The following salient points were presented during the lectures as well as in informal talks.

***Sirex* mass-rearing:**

It is necessary to have sufficient numbers of the *Sirex* host in the correct stage of development to present to imported parasitoid species both for quarantine purposes and for mass-rearing. The key to the rearing of *Sirex* is that anything that enhances the growth of its symbiotic fungus *Amylostereum areolatum* in effect promotes the growth of the wasp itself. There is an optimal range of moisture and temperature in which the fungus grows. Being a weak pathogenic fungus a tree must first be stressed before the fungus can become active and this is enhanced by the mucus which the wasp deposits in the tree during oviposition which causes further wilting.

Under ideal conditions the water lost through the crown of a tree is replaced from water via the roots and no tension exists within the tree. If the tracheids are cut this results immediately in tension as the water moves away from the cut area as a meniscus is formed. The same effect is caused when the mucus deposited by the *Sirex* wasp is sucked up in the sap stream into the foliage where it causes excessive transpiration resulting in the wilting of the needles and tension caused by a lack of water. A cut billet loses water from both ends where a meniscus forms but because the water is not drawn out of the log, the billet is often unsuitable for rearing *Sirex* larvae because it contains too much water. Usually the wasps probe such billets but rarely lay eggs and if these develop to maturity they are often very small and invariably male. This is because the fungus cannot flourish under these water-logged conditions.

Where the *Sirex* larvae feed on the fungus, the fungus is stimulated to grow (much like when mowing a lawn) and the nutrients are moved to the grazed area via the hyphae. This in turn results in the optimal growth of the *Sirex* larva. For the optimal rearing of *Sirex* larvae, host trees need to be felled during the active flight period and allowed to hook onto adjacent trees. This allows maximum air circulation around the tree and the adhering foliage results in moisture being removed from the stump by transpiration. Such trees become ideal hosts for *Sirex* oviposition. It is also possible to place laboratory reared *Sirex* wasps whose wings have been trimmed to prevent them flying, onto such logs. They will then move up and down depositing eggs. The only drawback here is that these woodwasps may be vulnerable to bird predators. Wasps could also be confined to parts of the tree by making use of nets. Once the tree is saturated with oviposition holes and the foliage has turned brown it may be cut into billets of ± 2 m in length whose ends are sealed with grease or wax to prevent further water loss, and brought back to the laboratory as hosts for the parasitoids.

A cut log is a static column of water and under these conditions there will be usually single drills only made by the wasps and if eggs are laid the emerging *Sirex* will be very small because a high water level is not conducive for the growth of the fungus. As the osmotic pressure drops the greater the number of eggs laid at each oviposition site (from 1 to 5 eggs) (Table 1.) As the tree deteriorates further the female *Sirex* chances laying more and more eggs into the tree so that the proportion of drills increases with time (Table 2).

Table 1.

Osmotic
pressure

1 2 3 4 5

Number of eggs laid
per oviposition site

Table 2.

Proportion
of drills

Time

As a tree dries out the water is removed from the top downwards so that a situation can exist where the wasps have already emerged from the top the previous season but have yet to emerge from the water saturated lower base. The tree may either have been attacked entirely by *Sirex* in the first season but the eggs in the lower trunk failed to hatch then because the water level was too high, or the lower trunk could have become attractive to the wasps only during the second season.

The reason why some *Sirex* wasps may take two years to emerge is largely determined by temperature. The lower the temperature the longer their development takes. In Brazil a life-cycle can be completed in 2 - 3 months when the eggs are laid in the stem of the crown and this is assumed to be a result of the faster drying out of the crown and less food available which speeds up the life-cycle.

If female *Sirex* wasps continue to orientate towards the lights in the laboratory it usually means they are not yet mated. Under natural circumstances the male wasps hover above trees waiting for females to appear but once mated the females become photonegative and then head downwards to lay eggs. Males usually orientate to the highest point and swarm there. They have a different flight sound to that of the females which sounds more like a flutter and its possible that the sexes use this sound in part to distinguish between them.

The hymenopterous parasitoids are attracted by smell to the trees which contain *Sirex* fungus. There is a wet area around the *Sirex* larva resulting from the faeces on which micro-organisms and fungal elements feed and this is exploited by bacteria and yeasts. The rhysines (*Megarhyssa* and *Rhyssa* species) find these "hot-spots" due to the high water and nitrogen levels found immediately behind the larva by antennal detection. The rhysines will lay an egg if its probe encounters such a wet spot.

Parasite handling and rearing:

The key requisites for rearing the parasitoids are honey, water and pollen. With sufficient nutrients *Megarhyssa* can live up to 13 weeks in the laboratory. In Australia *Megarhyssa* were observed to leave the plantation and visit flowers in the immediate surroundings of the plantation. *Rhyssa* females would also look for food even if they had already been mated and therefore not only for maturation feeding.

Then the parasitoids must be presented with adequate supplies of infested billets of the right stage of *Sirex* i.e. mature larvae for the rhysines and very young larvae (or eggs) for the ibaliids. For ibaliids this is best achieved by utilizing naturally conditioned methods of culture viz. the infestation of felled trees in the plantation, the use of tree cages confining *Ibalia* to either naturally infested trees or trap trees which had been attacked by *Sirex*.

Adult parasitoids must be protected at all times from extreme temperatures and any predator, especially spiders in cages, must be discouraged. The *Sirex* material to be parasitised must not be left too long with the parasitoids i.e. not longer than one week with a single female parasitoid.

Rhysines can be provoked to lay eggs through filter paper onto a wet surface on the other side. It is very important that the surface is wet or else the eggs will not be deposited. A globlet of venom will first be released to paralyse the larva and then the egg will be deposited. These eggs can then be transferred to honeybee larvae which are placed in a specially constructed "grid" system to complete their development which can be as fast as four days. To stimulate the rhysines to lay through the filter paper a wedge of wood containing some *Sirex* larval tunnels must be present. The behaviour of the ibaliids is different in that they will probe through the filter paper but will not deposit any eggs and so this method of mass-rearing is unsuitable for them.

Megarhyssa males have a pheromone produced from their pharyngeal glands which is species specific. Swarms of *Megarhyssa* males congregate on the bark around a site where a *Megarhyssa* male or female is about to emerge. The males arch their abdomens when waiting for females to emerge. This behaviour can also be stimulated if a piece of filter paper treated with either the female smell or *Sirex* odour is presented to them. In a similar way, the filter paper needed for the stimulation of the rhysines to lay through it can be prepared by placing it over the bark of logs containing *Sirex* tunnels. The odour from the bacteria around the posterior of the *Sirex* larvae will permeate the bark and be absorbed into the filter paper.

Ibalia, however, responds to the fungus which has been shown experimentally to be most attractive to them on the 10th day after it was deposited. *Ibalia* will therefore probe in tunnels which may only contain mucus and spores.

By experimentally marking female *Sirex* it was shown that there was very little movement from one tree to another if the first tree was suitable. This behaviour can be used to confine *Sirex* to suitable trees (by either clipping wings or by nets) in order to rear enough hosts of the correct stage. *Sirex* females on average lay 120 to 150 eggs. Blue-stain fungi were only a problem in the lab (where they are antagonistic towards the *Sirex* fungus) but were never a problem in nature. The presence of blue-stain fungus excludes *Sirex*. The polyphenols produced by the host tree inhibit the *Amylostereum* fungus in the field but it is only todays photosynthate which can be used to produce the defensive phenols to block the fungus i.e. cannot use stored synthate to produce these phenols. Hence the effectiveness of the mucus in preventing translocation of the synthate to areas where it is needed to contain the fungus.

Mass-rearing of *Ibalia leucospoides* in Brazil

Ibalia leucospoides was not introduced to Brazil but arrived by chance where about 30 % parasitism is now registered in the plantations with a maximum of 46 %. *Ibalia* is an endoparasite for the first two stages and part of the third stage as a larva but then bites its way out of its host and feeds on the *Sirex* larva from the outside. Ibalia are the only endoparasites which attack *Sirex*. The highest seasonal parasitism rate of 33 % is usually in September.

Ibalia adults live for 25 days at temperatures of 4, 8 and 12 °C but after this there is mortality (30 %) at 4 °C. *Ibalia leucospoides* can be stored at 12 °C for 30 days with a 100 % survival rate. At 35 days the male survival rate drops to 80 % but the females still remain at 100 %. The highest survival rate for *Ibalia* was when they were kept at 12 °C on 20 % honey water.

There are two emergence periods for both *Sirex* and *I. leucospoides* in Brazil. Rearing of *Ibalia* was shown to be better when they were recruited from the second emergence period because the parasites were larger and the survival rate much longer. *Ibalia* spends eight minutes laying one egg. For ideal mass-rearing of *Ibalia* they must be presented with billets 2 - 6 weeks old and kept at 25 °C and 70 % RH.

Dispersal capacity of the parasitoid species

Much of the success of introduced parasitoid species depends on their ability to disperse. The following dispersal distances were gleaned from the literature.

Ibalia leucospoides	New Zealand	6 km in 4 years
		125 km in 9 years
Megarhyssa nortoni	New Zealand	65 km in 9 years
		115 km in 5 years
	Tasmania	19 km in ± 12 years
Rhyssa persuasoria	New Zealand	>5 km in ± 20 years
	Tasmania	7.2 km in ± 12 years

Ibalia leucospoides was found to be the best parasite in Tasmania and Victoria. It was originally imported from Europe and Turkey. *Megarhyssa nortoni* was introduced from western Canada and so is not a natural parasitoid of *Sirex noctilio*. *Rhyssa persuasoria* was originally collected from Europe, Japan and Morocco. It was reported by the delegates from Chile that although *S. noctilio* has not yet arrived in that country, *Ibalia leucospoides* has already been recorded there on a related indigenous *Urocerus* species.

In Tasmania *Ibalia* has a lower effect on *Sirex* because both the trees and insects take a lot longer to develop due to the colder conditions. *Ibalia* holes are flooded with resin and their eggs are killed. This makes *Ibalia* density independent in Tasmania and therefore less effective. But *Ibalia* is density dependent (i.e. its numbers increase in response to an increase in host numbers) in South Australia where temperatures are warmer and is therefore much more effective there.

It is theoretically best to release the parasitoids where the host is in an expanding mode i.e. on the periphery, rather than where the host population has begun to crash due to, for example, the lack of suitable host trees.

There is usually a 5 - 6 year interval from the arrival of *Sirex* to the reaching of epidemic levels. It is best to introduce parasitoids when the population is low i.e. within the first 5 - 6 years. The introduction of the first biological control agent within the second season after the discovery of *Sirex* in South Africa is thus vindicated. Although the initial plan (for cost effective reasons) was to introduce only *Ibalia leucospoides* and *Megarhyssa nortoni*, the experts are adamant that *Rhyssa persuasoria* must also be introduced. *Rhyssa* parasitises *Sirex* larvae in narrow diameter logs and is therefore almost entirely confined to thick branches and the stem in the crown of the tree, while *Megarhyssa* parasitises larvae in larger diameter logs and is mostly found on the trunk. These two species in effect complement one another and both should be introduced.

Measuring parasitism rate

The parasitism rate in Australia was measured by placing cages at different heights on susceptible trees, each cage containing a *Sirex* female. The cages could be moved up or down as required. From this it was possible to tell how many eggs were laid by *Sirex* from the number of oviposition holes under the bark, and to determine how many of these eventually resulted in adult wasps. The parasitism rate was determined by removing the gauze and replacing it later after the parasites had had access to it. This method was used by Keith Taylor in his key factor analysis which he published.

Trap trees

Pinus radiata is well adapted to drought conditions and enters a quiescent period in summer. It responds very quickly to rains and achieves 80 % production in a short time. But when quiescent it is under high stress. This is when it is attacked by *Sirex.*

Trap trees can be set out 3 - 4 weeks before the *Sirex* flight season as long as they are heavily stressed and are dying fast. Treatments with Dicambra or Triclopyr at 2 cc per 10 cm of circumference can also be used. Tordon is also effective and appears to have no ill effect on *Sirex* larvae.

Prune trap trees to a convenient height and cut a girdle of 2 cm below the upper crown. With root grafting (i.e. flow of nutrients between the roots of adjacent trees) water will still move up the tree which will live for several weeks. Carbohydrates will come down the phloem as far as the girdle and the upper part of the trunk will increase dramatically in girth compared to the section below the girdle. Trap trees can also be enhanced for attraction by putting old bark around the dying tree. Scorching logs (there must be plenty of smoke) will also result in *Sirex* wasps being attracted within hours.

Experiments conducted in Brazil showed that the vast majority of *Sirex* eggs are laid in the middle third of the trunk. The base of the tree has few *Sirex* but the numbers increase dramatically in the centre (30 - 80 % of the tree), with a decrease in numbers as the trunk tapers. Madden confirmed that there is a "magic point" 30 % or one third of the way up the trunk where *Sirex* first begins to lay eggs. The high population levels of *Sirex* in Brazil result in up to 1800 *Sirex* emerging from a single 15 year old *P. taeda* whereas in South Africa only an average of 300 *Sirex* emerged from 44 year old *P. radiata* trees.

Additional information

Sharpness of punches: Dr. Madden suggested that we check the sharpness of our punches because the reason for our initial 23 % parasitism rate by *Deladenus siricidicola* might be due to the cauterizing of the tracheids. These punches were second hand when we bought them. Initial tests at Rosebank have shown that this is indeed the case where only ± 20 % of the tracheids in a punched hole are open.

Quarantine: Brazil has been largely able to circumvent quarantine and this has greatly assisted in getting the mass rearing of *Megarhyssa nortoni* going. They were supplied with a phytosanitary certificate from Australia which was acceptable to the Brazilians. It is unlikely that the South African officials will accept a similar arrangement. The mortality rate during importation was very high for these female parasitoids costing Aus. $ 100 each. In the first consignment of 70 females only 14 remained by the time they had reached the culture rooms at Curitiba 14 days after their introduction through Sao Paulo.

Deladenus wilsonii: This parasitic nematode was intercepted in early introductions of hymenopterous parasitoids into Tasmania. It sterilises both *Sirex* and the rhysine parasitoids and was excluded from becoming established because it would have a very negative effect on any such biological control programme.

Sirex activity peak: In Argentina there is a small emergence peak in January but most *Sirex* emerge in March. This is similar to South Africa and any importation of parasitoids from that country will be in synchrony.

Parasitism rate in Brazil: The *Deladenus* nematode imported originally from New Zealand in 1990 and then later the Kamona strain from Australia is now achieving an average of 54 % parasitism in southern Brazil although this can vary between 22 % and 99 % at different sites. *Ibalia leucospoides* also varies in its effectiveness between sites from 3.97 % to 18.82 %.

Eventual dispersion: In Brazil *Sirex* is presently in the south in a region which has rainfall all year around but is effectively sub-tropical. It was reaffirmed that *Sirex* is likely to inhabit all areas where its hosts can be grown.

Staff and equipment: It transpired that only in South Africa are the research staff responsible for the entire establishment of the *Deladenus* nematodes. In all other countries field workers from forestry companies were trained by the researchers to carry out the inoculations in their own plantations. Should the *Sirex* escape its present limits in the southwestern Cape and enter the plantations of the summer rainfall areas, the training of such personnel would also become necessary.

Funding: The Embrapa budget at Curitiba for both silvicultural research and forest protection was US $ 80 million per annum for a staff of 154. This funding was from central government alone. Their facilities were far superior to any of the forest protection units in South Africa and their equipment was the most modern available. The housing and cages erected to facilitate the importation of *Megarhyssa nortoni* and *Rhyssa persuasoria* was estimated to have cost R400 000. The overall impression was that the expertise present in South Africa is far superior to that in Brazil but the South African facilities and funding are a disgrace when compared to that of Brazil.

There were complaints by all organisations represented at the workshop over the exorbitant amounts being charged by the Australians for the purchase of their biological control agents. While these amounts may be exorbitant, to start from scratch collecting these agents in their natural habitats in Europe etc. would possibly cost more. Agreements were made in principle with individual South Americans to supply such parasitoids to South Africa at half price to offset their cost of importing them from Australia.

Acknowledgement:

My participation on this course was mainly funded by the Forest Owners Association and their assistance is gratefully acknowledged.

G. Tribe
Plant Protection Research Institute
Ryan Road, Rosebank 7700
Cape Town
Fax: 021 - 685 2017
Tel: 021 - 685 5661
E-mail: nipbjum@plant1.agric.za