Commercial in Confidence

Client Report No. 38510

Stem Injection - Operational Stem Injections trials and bioassays

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THE JOINT FORCES OF CSIRO & SCION

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Date: **July 2005**
Client: **Forest Biosecurity Research Council (FBRC) and Ministry of Agriculture and Forestry (MAF)**
Contract No:

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EXECUTIVE SUMMARY

Six insecticides were evaluated for their efficacy to control herbivorous insects on *Eucalyptus* species in the greater Auckland region of New Zealand. Insecticides were injected by force directly into the xylem of *Eucalyptus cinerea, E. saligna* and *E. globulus*. Two insect species, *Uraba lugens* (Gum Leaf Skeletoniser) and *Paropsis charybdis* (Eucalyptus Tortoise Beetle) were used in a series of eight operational injection trials and 34 bioassays.

Foliage from injected trees was randomly sampled and used in bioassays. Tamaron, an organophosphate insecticide, proved to be highly successful in all bioassays on *Uraba lugens* and one on *Paropsis charybdis.* Tamaron treatments consistently inflicted more then 95% mortality on target insect larvae. Effective insect control was achieved within 2 days after injection and lasted beyond 128 days. None of the other selected insecticides achieved sustained or acceptable insect larval control.

OBJECTIVE

This final phase of the project focused on finding operational solutions to controlling herbivorous insects feeding on a variety of Eucalyptus species, in the greater Auckland region of New Zealand. The main objectives of these trials were to determine if the injected insecticides would successfully translocate from the xylem into the foliage and to determine the persistence of the pesticide in the tree.

Results obtained from the stem injection dose response trials conducted on *Eucalyptus nitens* (phase III) in Rotorua were used as guidelines for the treatments used in this phase. It needs to be emphasized that this phase ran concurrently with phase III due to time constraints with insect life cycle and availability. Trees infested with *Urba lugens* larvae were selected for injection. Foliage from injected trees was sampled and used in bioassys using the methods described in phase III (Gous 2005).

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Information for Ensis abstracting:

Contract number	
Client Report No.	38510
Products investigated	Methamidophos, Deltamethrin, Imidacloprid, Thiacloprid and Spinosad
Wood species worked on	*Eucalyptus globulus, E. cinerea, E. saligna*
Other materials used	Stem injectors
Location	Puketutu Island, Auckland airport golf course

MATERIALS AND METHODS

Foliage sampling

Foliage samples were randomly collected from the branches of injected trees, 2m above ground. Samples were placed in plastic bags, labelled and kept under refrigeration at 4 ^{O}C until used for the bioassays. Where possible, only mature foliage was used.

Pottle system

In all bioassay trials, larvae were kept in opaque plastic drinking cups with experimental host plants. A twig with leaves was placed in a shorter cup (395 ml) with a hole in the bottom. The stem of the twig wrapped in a piece of paper towel was placed through the hole, and the smaller cup was fitted inside a longer cup (595 ml) with water in the bottom, allowing the stem to be immersed in water (Matsuki *et al*., 2001). Five larvae from the chewing stage were then placed in each cup, with four replicate cups per treatment (figure 1).

Figure 1. Uraba lugens caterpillars on Eucalyptus nitens leaves in pottle sytem.

Unless otherwise specified, all bioassays were conducted using *Uraba lugens* larvae (figure 2a) reared in the Ensis quarantine facility using the pottle system. *Paropsis charybdis* larvae (figure 2b) were used in some direct comparison bioassays with *Uraba lugens*. Insect larvae were monitored for mortality and pupation at 96 and 168 hours exposure. Bioassays were kept in a double containment system as seen in figure 3.

Figure 2a **Uraba lugens** ***larva***

Figure 2b **Paropsis charybdis** ***larva***

Figure 3. Double containment system used for all **Uraba lugens** ***bioassays.***

Stem injection – methods and equipment

With the exception of one trial, all the injections were done by a forced low volume injection, using the “Stemject” injector (figure 4). This system uses a 8mm hole drilled into the tree trunk. The injector nozzle is screwed into the hole, sealing tightly (figure 5) before pressure is applied. Insecticides are injected directly into the xylem by force, ensuring that the injection pressure does not exceed 3 bars. This type of injector is very versatile and application volumes ranging from 5ml to approximately 150ml can be injected into a single hole.

Figure 4. The Stemject injector.

Figure 5. Stemject injector nozzle sealed into injection hole.

The only other injection method used was a passive low volume injection, used in one of the Tameron injection trials. In this method a hole is drilled at a downward sloping angle of approximately 30 degrees (figure 6). The required volume of insecticide is then placed into the hole by syringe. This method is very accurate, but extreme care must be taken not to exceed the volume of the drilled cavity. Multiple injections can be made into the same hole if required as soon as the insecticide has been taken up into the xylem.

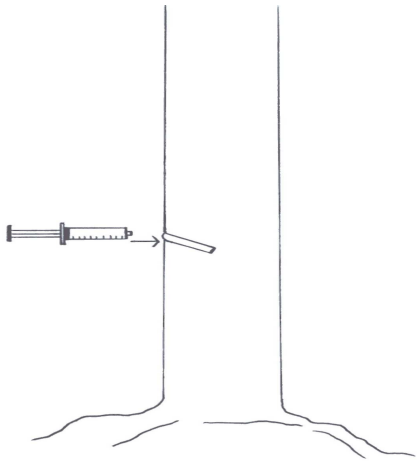

Figure 6. Passive low volume injection.

Stem injection - dye injection

Fifty ml of water soluble blue marker dye was injected into the base of each of two very large (27m tall) *Eucalptus grandis* trees. The first tree was felled 90 minutes after injection, the second tree 4 days after injection. The stems were crosscut at 25cm intervals to determine the fate of the injected marker dye. Ninty minutes after injection, the dye had moved to 7m above the injection point. Four days after injection, the dye had been translocated to the top of the tree. Figures 7 to 12 taken 4 days after injection show the translocation path, at 5m intervals. It is noticeable that the dye mainly moved in the sapwood. Lateral spread increased with height (distance) from the injection point to the full tree circumference at 20m above the injection (figure 11).

Figure 7. Point of injection.

Figure 8. 5m above injection point.

Figure 9. 10m above injection point.

Figure 10. 15m above injection point.

Figure 11. 20m above injection point.

Figure 12. Branch at 19m above injection point.

Stem injection bioassays

Injected insecticide rates were calculated by using tree or branch diameter as an indicator to determine the volume to be injected. Injected insecticide rate-ratios were kept between approximately 0.5 – 5.0 of the diameter for all selected trials. Insecticide rate was calculated as ml insecticide per cm tree stem diameter.

Tamaron – Methamidophos

Tameron trial 1, *Eucalyptus cinerea*: Four trees growing at the Auckland Airport Golf course were treated. These were old and very large trees, therefore branches were selected for injection, rather than the stem. *Eucalyptus cinerea* has very thick (40mm - 75mm), fibrous bark. At the location of the injection, the bark was removed prior to injection to ensure that the injector nozzle would seal against the stem. Rates selected for injection were 2, 3, 3.5 and 4 times the diameter.

All selected branches were infested with *Uraba lugens* larvae. Large pillows of curtain net were placed around selected branches. *Uraba lugens* larvae responses were monitored. Foliage samples were collected at 2, 15 and 31 days after injection and used in pottle bioassays with *Uraba lugens* larvae. Foliage collected 15 days after injection was also trialed in a pottle bioassay using *Paropsis charybdis*.

Tameron trial 2, *Eucalyptus globulus* large trees: Stems of four trees growing on Puketutu Island were injected. Tree sizes ranged from 25cm to 29cm diameter. Rates selected for injection were 0.5, 2.0, 3.0 and 3.5 times the diameter ratio. Branches with *Uraba lugens* egg batches were selected and covered with large pillows of curtain net to prevent larvae from escaping once hatched. *Uraba lugens* egg masses were monitored on the foliage. Foliage samples were collected seven times after injection at 7, 15, 31, 38, 45, 57 and 70 days post injection and used in pottle bioassays with *Uraba lugens* larvae.

Tameron trial 3, *Eucalyptus globulus* small trees: Five small trees, ranging from 11cm to 17cm were selected for a passive injection trial. One 10mm diameter hole per tree, was drilled into the stem at a downward slope of approximately 30 degrees. Seven ml of Tameron was injected by syringe into each drilled cavity. Therefore rate ratios were determined by the size of each tree. Foliage was collected three times after injection and used in pottle bioassays on Uraba lugens larvae.

Decis Forte – Deltamethrin

Decis trial 1, *Eucalyptus saligna*: Stems of four trees on Puketutu Island were injected with Decis. Tree size ranged from 24cm to 34cm diameter. Rates selected for injection were 1.25, 1.75, 2.0 and 3.0 times the diameter ratio. Foliage was collected at 4 and 15 days after injection for bioassays. *Uraba*

lugens and *Paropsis charybdis* were used in pottle bioassays on foliage collected 4 days after injection. Only *U. lugens* was used in the 15 day after injection bioassay.

Decis trial 2, *Eucalyptus globulus*: Three small trees ranging in size from 10cm to 15cm were injected with Decis on Puketutu island. Rates selected for injection were 2.0, 2.6 and 3.0 times the diameter ratio. Foliage was collected and used in pottle bioassays on *U. lugens,* 7, 15 and 31 days after injection.

Calypso –(Thiacloprid), Confidor –(Imidacloprid), and Success Naturalyte –(Spinosad).

Three insecticides trial 1, *Eucalyptus globulus*: All three insecticides were used in one injection trial on Puketutu Island, Auckland. The negative results encountered from these insecticides in phase III of this project was probably due to an inability for the insecticides to be translocated through the vascular system of the trees. This could be caused by two factors. Adjuvants in all of these formulations are designed to assist the active ingredients to spread and stick to the plant surface onto which it is sprayed. The formulations also have very low water solubility, which further decreases the chances of them being translocated to the foliage. Therefore, it was decided to extract the active ingredients from the formulated products and re-suspend the active ingredient in 50ml distilled water for injection.

Each insecticide was injected at 2 and 3 times the rate ratio of the tree diameter. Only the higher rates were initially used in bioassays to determine efficacy. Six bioassays were conducted with *U. lugens* at 7, 15, 23, 31, 39 and 53 days after injection.

Confidor Supra trial 2, *Eucalyptus globulus*, large trees: It was decided to use a different formulation of Imidacloprid emulsifiable concentrate (EC) for the next set of injection trials, due to the low solubility of the suspension concentrate (SC) formulation. The EC was not used initially because it contained a secondary synthetic pyrethroid in the formulated product.

Four similar sized, large trees, (23cm to 28cm diameter) were selected for injection at 2.0, 3.0, 4.0 and 5.0 times the tree diameter rate ratio.

Foliage samples were collected four times at 10, 17, 31 and 48 days after injection and used in pottle bioassays on *Uraba lugens* larvae (Table 7).

Confidor Supra trial 3, *Eucalyptus globulus*, small trees. This trial ran concurrent with trial 2, above. Six similar sized, small trees, (7cm to 14cm diameter) were selected for injection at 3.0, 3.5, 4.0, 4.5, 5.0 and 5.5 times the diameter rate ratio. Foliage samples were collected four times at 5, 12, 26 and 43 days after injection and used in pottle bioassays on *Uraba lugens* larvae (Table 8). Chemical analysis was done with foliage collected 2m above

ground level from trees injected with the two highest rates, 50 days after injection, in an attempt to determine if the active ingredient was present.

RESULTS and DISCUSSION

Stem injection bioassays

Tamaron – Methamidophos

Tameron trial 1, *Eucalyptus cinerea*: *Uraba lugens* larvae inside the net pillows placed over branches injected with Tameron, showed a very high pupation rate of approximately 85%, compared to the control's approximately 35% pupae, 15 days after injection. The remaining 15% of the caterpillars died within 10 days after injection. Branches injected with the high Tameron rates appeared to shed much higher numbers of leaves compared to the untreated surrounding branches.

Translocation of Tameron did not occur within the first two days after injection (Table 1). However, 15 days after injection both *Uraba lugens* and *Paropsis charybdis* larvae were controlled very effectively. The third bioassay done 31 days after injection showed a rapid decline in efficacy as seen in table 1. It should be noted however, that the quality of the foliage had already deteriorated by this time, possibly due to the high concentration of Methamidophos. Insect larvae preferred not to feed much on this foliage and pupation rates were high, ranging from 40% - 60%.

Table 1. *Uraba lugens* and *Paropsis charybdis* mortality* (%) response to four rates of Tameron forcibly injected into *Eucalypts cinerea* at Auckland airport golf course

Species	Days after injection	Rate ratio injected (ml/cm)				Control
		2.0	3.0	3.5	4.0	
Uraba	2	0	0	0	0	0
Uraba	15	100	100	100	100	10
Paropsis	15	100	100	100	90	10
Uraba	31	35	75	100	15	5

** Insect mortality percentages calculated after 1 week's exposure to foliage from trees injected with Tameron*

Tameron trial 2, *Eucalyptus globulus* large trees: Effective translocation of Tameron did not occur within the first 15 days after injection. However, 31 days after injection *Uraba lugens* larvae were controlled very effectively by the two higher rates. This effect lasted beyond 70 days after injection (Table 2). The lowest rate of 0.5 times the diameter is probably too low to effectively control *Uraba lugens* larvae. A rate of 2.5 times the diameter, also showed very low efficacy in controlling the *Uraba* larvae. All previous results at similar rates provided very high levels of control. In most cases, mortality well above 90% was achieved. The relative poor efficacy of this injection can't be explained. It is the only Tameron injection that showed unsatisfactory control of *Uraba lugens* in phases III and IV of this project.

Table 2. *Uraba lugens* mortality* (%) response to four rates of Tameron forcibly injected at Puketutu Island, Auckland on *Eucalypts globulus*

Days after injection	Rate ratio injected (ml/cm)				Control
	0.5	2.5	3.0	3.5	
7	0	0	15	25	5
15	20	0	40	55	20
31	65	15	100	100	0
38	55	30	100	100	5
45	40	0	100	90	0
57	30	10	100	100	5
70	10	10	100	100	15

** Insect mortality percentages calculated after 1 week's exposure to foliage from trees injected with Tameron*

Tameron trial 3, *Eucalyptus globulus* small trees: There is little doubt that passively injected Tameron had been translocated by the tree, when mortality rates are compared with the control. Translocation is marginally slower than for forced injections, as indicated by the mortality rates one month after injection. Mortality rates indicate that Tameron injected in this fashion is also a highly effective option to control *Uraba lugens* larvae, considering that very low insecticide rates were used. However, this method of injection is not recommended if a forced injection option is available, as a relatively large wound is caused by the 10mm drilled hole. Forced injections allow higher insecticide volumes to be injected through much smaller holes in the tree. Translocation is marginally faster than in passively injected trees.

Table 3. *Uraba lugens* mortality* (%) response to five passively injected rates of Tameron, on *Eucalypts globulus*

Days after injection	Rate ratio injected (ml/cm)					Control
	0.58	0.44	0.41	0.54	0.64	
14	40	5	10	30	10	0
31	65	65	20	65	55	10
80	90	75	35	85	100	10

** Insect mortality percentages calculated after 1 week's exposure to foliage from trees injected with Tameron*

Decis Forte – Deltamethrin

Decis trial 1, *Eucalyptus saligna*: *Paropsis charybdis* appeared to have very little tolerance to the injected Decis (Table 4). At the two higher rates, 80% and 95% mortality was recorded for *P. charybdis* compared with only 5% and 15% mortality respectively for *Uraba lugens.*

At the injected rates, Decis did not control *U. lugens* larvae effectively. Furthermore, there appears to be no relationship between the injected rates and percentage mortality.

Table 4. *Uraba lugens* and *Paropsis charybdis* mortality* (%) response to four rates of Decis injected into *Eucalypts saligna*

Species	Days after injection	Rate ratio injected (ml/cm)				Control
		1.2	1.7	2.0	3.0	
Paropsis	4	20	25	95	80	35
Uraba	4	5	0	5	15	0
Uraba	15	35	45	20	20	5

Decis trial 2, *Eucalyptus globulus*: The results from the bioassays clearly indicate that very little translocation of Deltamethrin had occurred (Table 5). Based on this, and results from other trials, Decis was not tested in further injection trials.

Table 5. *Uraba lugens* mortality* (%) response to three rates of Decis injected at Puketutu Island, Auckland on *Eucalypts globulus*

Days after injection	Rate ratio injected (ml/cm)			Control
	2.0	2.6	3.0	
7	0	0	0	0
15	15	0	5	5
31	10	15	5	0

Calypso –(Thiacloprid), Confidor –(Imidacloprid), and Success Naturalyte –(Spinosad).

Three insecticides trial 1, *Eucalyptus globulus*: Results from this injection trial (Table 6) indicate that none of the injected insecticides had been translocated. The most likely reason is that the active ingredients of all three insecticides are highly insoluble in water and therefore were not translocated much beyond the point of injection. The use Calypso or Success Naturalyte in further injection trials was discontinued.

Table 6. *Uraba lugens* mortality* (%) response to one injected rate (3 ml/cm) of Calypso, Confidor and Success Naturalyte

Days after injection	Calypso	Confidor	Success	Control
7	0	0	0	0
15	0	0	15	0
23	5	0	5	0
31	0	5	0	5
39	0	5	0	0
53	10	5	0	0

Confidor Supra trial 2, *Eucalyptus globulus*, large trees: *Uraba lugens* mortality on foliage from trees injected with Confidor Supra was very low, with only 0-10% mortality over the control in any bioassay (Table 7). These results

are very similar to previous trials with Confidor. Results suggest that very little translocation of either the Imidacloprid or the secondary synthetic pyrethroid had taken place, even after seven weeks.

Table 7. *Uraba lugens* mortality* (%) response to four injected rates of Confidor Supra, on *Eucalypts globulus*

Days after injection	Rate ratio injected (ml/cm)				Control
	2.0	3.0	4.0	5.0	
10	10	0	0	5	0
17	10	0	0	0	0
31	20	15	25	25	15
48	20	15	25	25	15

Confidor Supra trial 3, *Eucalyptus globulus*, small trees. Six weeks after injection, there was little evidence that the insecticides had been translocated into the foliage of the injected trees (Table 8). Chemical analysis of the two highest injected rates was unable to detect either of the two injected insecticides 50 days after injection.

The largely negative results (Tables 7 and 8) from the trials using the EC formulation of Imidacloprid indicate that it was not the adjuvants of the original SC Imidacloprid formulation that caused the lack of translocation. Trees should ideally be monitored over time to see if translocation does occur.

Table 8. *Uraba lugens* mortality* (%) response to six injected rates of Confidor Supra, on *Eucalypts globulus*

Days after injection	Rate ratio injected (ml/cm)						Control
	3	3.5	4.0	4.5	5.0	5.5	
5 days	0	0	5	0	0	0	0
12 days	0	0	15	0	0	10	0
26 days	5	10	10	10	10	10	15
43 days	5	0	5	5	10	10	25

RECOMMENDATIONS AND CONCLUSIONS

Efficacy, as determined by mortality of the target insect in bioassays, was dependant on insect species, insecticide type and injected insecticide rate. Direct injection of insecticides to control herbivorous insects in an urban environment proved to be a viable option. Further work to optimise rates and injection methods should be considered. Insecticide persistence and tree wound recovery should also be further investigated and monitored.

Tamaron – Methamidophos

- In most cases Tameron was translocated reasonably fast (within 2 - 7 days after injection) into the foliage from a stem injected application.
- Tameron was highly successful in controlling both *Uraba lugens* and *Paropsis charybdis* as seen from the results of the three sets of bioassay trials, tables 1, 2 and 3. Therefore Tameron can be recommended as a stem injected treatment to control herbivorous insects on *Eucalyptus* species.
- Bioassays conclusively indicated that Tameron was still 100% lethal at 2 months after injection on *Eucalyptus globulus*. *E. nitens* trees injected with Tameron (phase III) similarly showed 95% insect mortality 2 months after injection.
- Passive low volume injections of Tameron can be used to control insects on smaller trees, but relatively large drill wounds are caused.

Decis Forte – Deltamethrin

- *Paropsis charybdis* was much more sensitive to Decis than *Uraba lugens* larvae.
- There is some evidence that Decis was translocated into the foliage of stem injected trees.
- Stem injected Decis caused elevated mortality rates of both *Paropsis charybdis* and *Uraba lugens* larvae above the control treatments. However, mortality rates never reached acceptable levels to be recommended as an effective stem injection treatment.
- Results were variable with little signs of a dose related effect in controlling the skeletonising *Uraba lugens* larvae.

Confidor – Imidacloprid

- The initial results from stem injected Confidor (SC) suggested that very little translocation of the active ingredients had taken place. In all bioassays larvae mortality was very low and seldom higher than the controls. It was thought that the adjuvants in this formulation (SC) might contribute to the inability for the tree to translocate the Imidacloprid, active ingredient.
- The second formulation (EC) of imidacloprid similarly showed very little evidence that imidacloprid was translocated at rates lethal to *Uraba lugens* larvae, therefore neither Confidor, nor Confidor Supra can be recommended for stem injection to control herbivorous insects on *Eucalyptus* species.

Calypso - Thiacloprid and Success Naturalyte – Spinosad

- Two trials in phase III of this project revealed that both these insecticides had limited efficacy against controlling herbivorous insects.
- The extraction of the active ingredients from the formulated products, similarly had minimal effect on insect mortality, causing less than 20% mortality.
- Neither of these insecticides could be recommended to control herbivorous insects by stem injection methods.

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