Commercial in Confidence

Client Report No. 38511

Stem Injection - Dose response trials and bioassays

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Client: **Forest Biosecurity Research Council (FBRC) and Ministry of Agriculture and Forestry (MAF)**

Contract No:

EXECUTIVE SUMMARY

This phase of the stem injection project screened insecticides for uptake and efficacy. Six insecticides were evaluated for control of herbivorous insects on Eucalyptus species. Insecticides were injected by force directly into the xylem of *Eucalyptus nitens*. Two insect species, *Uraba lugens* (Gum Leaf Skeletoniser) and *Paropsis charybdis* (Eucalyptus Tortoise Beetle) were used in a series of 41 bioassays.

Foliage was sampled at approximately 7m above ground and used in the bioassays. Methamidophos an organophosphate insecticide, proved to be highly successful in 14 bioassays on *Uraba lugens* and one on *Paropsis charybdis.* Methamidophos treatments repeatedly inflicted more then 95% mortality to the insect larvae. Effective insect control was achieved within 24 hours after injection and lasted beyond 128 days after injection. None of the other selected insecticides achieved sustained and acceptable insect larval control.

OBJECTIVE

A series of trees were injected with insecticides. Foliage of these trees was then collected at various intervals after injection and used in dose response trials on *Uraba lugens* and *Paropsis charybdis*. The main purpose of these trials were twofold, firstly to determine if injected insecticides were translocated through the tree into the foliage, and secondly to quantify the rate required to successfully control herbivorous insects.

Initial results from the chemical analysis indicated that some of the injected insecticides translocated into the foliage of *Eucalyptus nitens* within 24 hours. The detectable insecticide concentration rapidly declined to about half of the original detected rates after 48 hours. By 72 hours after injection the level of insecticides had declined to almost undetectable levels. Therefore initial bioassays with foliage from injected trees were carried out within 6 to 48 hours post injection.

To judge success and efficacy, a mortality threshold had to be set. The general consensus was that for this type of application a minimum of 80% larval mortality would be acceptable, but higher mortality rates would be desirable.

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Information for Ensis abstracting:

Contract number	
Client Report No.	38511
Products investigated	Methamidophos, Deltamethrin, Imidacloprid, Thiacloprid and Spinosad
Wood species worked on	*Eucalyptus nitens*
Other materials used	Stemject tree injector
Location	Kapanga Hardwoods Tree Farm

MATERIALS AND METHODS

Stem injection

Three different stem injection methods were tested, passive high volume (PHV), passive low volume (PLV) and a forced low volume (FLV) system.

Passive high volume injection

For this system the required insecticide dose is diluted in 1 to 5 litres of distilled water. The system uses a high volume (1 -5 litre capacity) reservoir connected to a tapered nozzle via a flexible tube (figure 1). The nozzle is placed into a pre-drilled hole in the tree truck. The reservoir is strapped to the tree above the injection hole. The system is gravity fed, utilising atmospheric pressure only to "force" the liquid into the stem.

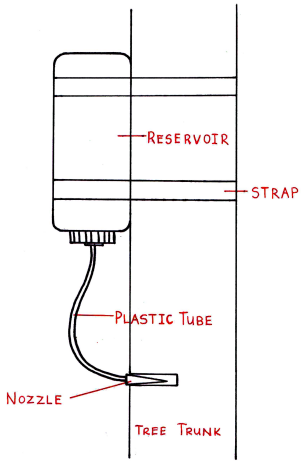

Figure 1. Passive high volume injection.

Passive low volume injection.

This application method is used to inject volumes below 10ml per injection point. A hole is drilled at a downward sloping angle of approximately 45 degrees (figure 2). 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 into the xylem.

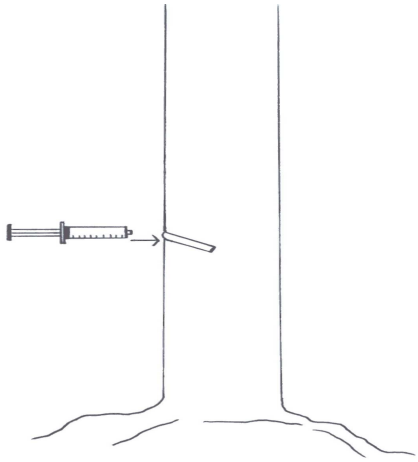

Figure 2. Passive low volume injection.

Forced low volume injection

A relatively small hole (and therefore wound) is drilled into the tree trunk. All these injectors have nozzles that screw into the hole, sealing tightly. Insecticides are then injected directly into the xylem by force. This type of injector is very versatile and volumes ranging from 5ml to approximately 150ml can be injected into a single injection point.

The “Stemject” forced low volume injection system (figure 3) became the preferred standard method of injection for this phase of the work. Unless stated otherwise this injector was used in all the forced injecting trials.

Figure 3. The Stemject injector.

Foliage collection

Foliage samples were randomly cut from the branches at approximately 7m above ground level. Samples were placed in plastic bags, labelled and kept

under refrigeration at 4°C until processed for the bioassays. Where possible, only mature foliage was used in all bioassays.

Pottle system

In all bioassay trials, treated plant stems were transferred into two-pot test arenas (535 ml), where the stem of the foliage was held in water and separated from the upper test chamber (395 ml) by a dense paper towelling bung (the same method used in previous bioassays, Matsuki *et al*., 2001). We then placed 5 larvae from the chewing stage in each cup, with four replicate cups per treatment (figure 4).

Figure 4. Uraba lugens caterpillars on Eucalyptus nitens leaves in pottle sytem.

Unless otherwise specified all bioassays were conducted using *Uraba lugens* larvae artificially reared in the Ensis quarantine facility using this pottle system. Insect larvae were monitored for mortality and pupation at 96 and 168 hours. Bioassays were kept in a double containment system as seen in figure 5.

Figure 5. Double containment system used for all Uraba lugens bioassays.

Pilot trials

Prior to stem injection application, the selected insecticides were tested on *Uraba lugens*. Two trials were conducted, a foliar insecticide application and a stem uptake application.

Foliar application trial:

Eucalyptus nitens branches were sprayed by knapsack sprayer with the manufacturers' recommended foliar application rates (table 1). Foliage was allowed to dry before larvae were placed onto it. Four replicates, each containing 5 larvae were used in a pottle trial to test insect sensitivity to the insecticides.

Stem uptake trial:

Stems of *Eucalyptus nitens* branchlets were placed in the recommended spray mixture (table 1) for 24 hours to allow uptake via the cut stem. Thereafter insects were placed on the foliage using the pottle system for bioassays.

Table 1. Insecticides rates used for foliar application against Uraba lugens. (Novachem Manual 2004/2005, New Zealand Agrichemical Manual 2002)**.**

Product commercial name	Active ingredient / litre product	Foliar and stem applied rates	Manufacturers recommended rates
Calypso	480g Thiacloprid	0.3ml/l	30ml/100litres, apply at maximum of 600 litres water per ha.
Confidor	350g Imidacloprid	3.6ml/l	9ml/2.5litres, apply at 500-600 litres water per ha.
Confidor Supra	75g Imidacloprid & 25g cyfluthrin	2ml/l	1 litre/ha, apply at 500-600 litres water per ha.
Decis Forte	27.5g Deltamethrin	0.4ml/l	36 - 40ml/100 litres, apply at 500 - 600 litres water per ha.
Success Naturalyte	120g Spinosad	0.4ml/l	40ml/100 litres, apply at 1000 litres water per ha.
Tamaron	600g Methamidophos	3ml/l	1 – 1.5 litres/ha in 500 litres water per ha.

Stem injection bioassays

Injected insecticide rates were calculated by using tree diameter at breast height (DBH) as an indicator to determine the volume to be injected. Injected insecticide rate ratios were always kept between 0.5 – 3.0 of the DBH for all selected trials. Example: To inject a rate ratio of 2 for a tree with DBH of 10cm, 20ml insecticide would be injected; similarly 5ml injected would result in a rate ratio of 0.5.

Tamaron – Methamidophos

Tameron trial 1: For the first stem injection trial, 2 trees of similar DBH and height were selected. The injected rate was double that of the diameter (16cm). Chemical analysis suggested that methamidophos concentration peaked within 24 hours, was reduced to half of the peak concentration within 48 hours and was virtually undetectable after 72 hours.
Therefore, foliage was collected 24 hours after injection for the first bioassays. Both *Uraba lugens* and *Paropsis charybdis* were used in separate bioassays. Larval mortality was monitored 24, 48, 72 and 168 hours after exposure.

Tameron trial 2: Five *Eucalyptus nitens* trees of similar DBH and height were injected with 10ml, 20ml, 30ml, 40ml and 50ml Tameron. Nine bioassays were conducted over a two month period at 1, 2, 8, 15, 22, 29, 43, 62 and 128 days post injection. The objective of this trial was to determine the minimum rate required for effective control and to determine the persistence of the insecticide in the plant tissue.

Tameron trial 3: Tameron was the only insecticide used in passive low volume injections. The use of this method was made possible due to the low volumes (10ml – 30ml) required for effective insect control as determined from the second stem injection trial.
Three *Eucalyptus nitens* trees of similar DBH and height were injected with 10ml, 20ml and 30ml Tameron. Four bioassays were conducted at 1, 3, 71 and 81 days post injection. The reason for the delay between bioassays 2 and 3 was that it was initially thought that this injection method was unsuccessful. However results from the other trials using Tameron showed prolonged control, therefore further bioassays were conducted.

Decis Forte – Deltamethrin

Decis Trial 1: Two trees of similar DBH and height were selected and injected by force at a rate ratio of 2:1 to that of the diameter. Detection of Deltamethrin in the foliage was very difficult and could not be replicated successfully. Chemical detection by HPLC was only achieved once. These results suggested that Deltamethrin was present in the foliage within 24 hours, but after 48 hours became undetectable by HPLC. Therefore, foliage of the injected trees were collected 24 and 48 hours hours after injection for

bioassays. Both *Uraba lugens* and *Paropsis charybdis* was used in separate bioassays. Larval mortality was monitored 24, 48, 72 and 168 hours after exposure. A third bioassay was done with foliage from the same trees, 30 days after injection.

Decis Trial 2: Five *Eucalyptus nitens* trees of similar DBH and height were injected with 10ml, 20ml, 30ml, 40ml and 50ml Decis. Seven bioassays were conducted at 1, 7, 14, 21, 47, 55 and 98 days post injection over a three month period. [Results from the first trial suggested that the injected rates might have been too low and that translocation was slow. Therefore additional time should be allowed, post injection, for effective translocation to occur.]

Confidor – Imidacloprid

Confidor Trial 1: Two trees were injected by force at a rate ratio of 2:1 to that of the diameter. During the injection of the first tree, the insecticide was not flowing freely into the xylem. Much higher pressure was required to inject the liquid insecticide. Due to the difficulty experienced in injecting the first tree, the second tree was injected with imidacloprid diluted 1:2 with water. This injection required much less pressure to inject than the undiluted confidor. Although a relatively high volume of 150 ml was injected it proceeded without any further difficulty.

Four bioassays with *Uraba lugens* were conducted with foliage collected 1, 2, 17 and 31 days post injection. Larval mortality was monitored 96 and 168 hours after exposure.

It is important to mention that prior to this trial, the active ingredient, imidacloprid was extracted from the formulated product, dissolved in water and injected in the operational trials. Results from these injections were largely negative and not repeated in these trials. Therefore a different formulation (emulsifiable concentration) of imidacloprid was sourced and used in all subsequent Confidor injections.

Confidor Trial 2: Five *Eucalyptus nitens* trees of similar DBH and height were injected with 10 ml, 20 ml, 30 ml, 40 ml and 50ml Confidor Supra. Five bioassays were conducted over a two month period at 8, 15, 25, 35 and 66 days post injection. The main objective of this trial was firstly to determine if the imidacloprid would be successfully translocated through the tree into the foliage. Secondly to determine an approximate rate required for successful *Uraba lugens* chewing larvae control.

Confidor Trial 3: Four *Eucalyptus nitens* trees of similar DBH and height were injected with 60ml, 70ml 80ml and 90ml of Confidor Supra. Four bioassays were conducted at 4, 14, 24, and 55 days post injection. The main objective of this trial was to determine if the higher imidacloprid rate would be successfully translocated, to reach lethal concentrations in the foliage.

Calypso - Thiacloprid and Success Naturalyte – Spinosad insecticides

Trial 1: Two trees each were injected by force at a rate ratio of 2:1 to that of the diameter. During initial pilot trial injections with Calypso and Success it was noticed that the insecticides were not flowing freely into the xylem, similar to the effect experienced with the initial Confidor injections. A much higher pressure was required to force the liquid insecticides into the tree. Therefore it was decided to dilute both insecticides with 1 part insecticide to two parts water. These diluted insecticides still proved to be reasonably difficult to inject. On two occasions the tree trunk ruptured and the injected liquid squirted out. Both these trees were not included in the trials, as it was impossible to determine the volume successfully injected.

Four bioassays with *Uraba lugens* were conducted with foliage collected 1, 2, 14 and 30 days post injection. Larval mortality was monitored 96 and 168 hours after exposure.

RESULTS

Pilot trials

Foliar application trial:

The five selected insecticides were highly effective against *Uraba lugens* chewing larvae although Tameron took longer to achieve acceptable mortality (Table 2).

Table 2. Larval mortality (%) from foliar application of insecticides.

Treatment	24hours	48 hours	72 hours
Control	0	10	10
Calypso	85	100	100
Confidor	100	100	100
Decis	100	100	100
Success	70	100	100
Tameron	40	50	95

Stem uptake trial:

Tameron was the only insecticide that produced 100% *Uraba lugens* larval mortality using the stem uptake method (Table 3).

Table 3. Stem uptake larval mortality (%).

Treatment	24hours	48 hours	72 hours
Control	10	10	10
Calypso	15	15	35
Confidor	60	60	70
Decis	5	5	10
Success	5	5	45
Tameron	95	95	100

Stem injection bio - assays

Tamaron – Methamidophos

Tameron trial 1: All *Uraba lugens* larvae were killed within 24 hours of exposure to the foliage taken from trees injected with Tameron (Table 4). On examination of the foliage very little feeding evidence was found.
Tameron was also highly effective against *Paropsis charybdis*, causing 95% larval mortality after 168 hours' exposure.

Table 4. *Uraba lugens* and *Paropsis charybdis* mortality (%)* after 24, 48, 72 and 168 hours of exposure to *Eucalyptus nitens* foliage from trees forcefully injected with Tameron.

Treatment	Larval mortality			
	24 hours	48 hours	72 hours	168 hours
Control *Uraba*	0	0	0	0
Uraba lugens	100	100	100	100
Control *Paropsis*	0	0	0	5
Paropsis charybdis	55	80	90	95

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

Tameron trial 2: A total of 1080 *Uraba lugens* larvae were used in nine sets of bioassays. Results from this trial (table 5) confirmed that Tameron was highly effective. Tameron was translocated into the foliage at lethal concentrations within 24 hours after injection. Efficacy of the lower injected rates only started to decline 6 weeks after injection. At the three higher injected rates Tameron was still 100% lethal 2 months after application. Results from these trials prompted the use of this product in the first operational trials in Auckland (Phase IV) on other target plant host species.

Table 5. *Uraba lugens* mortality (%)* response to five forcefully injected rates of Tameron

Days post injection	Tameron (10ml)	Tameron (20ml)	Tameron (30ml)	Tameron (40ml)	Tameron (50ml)	Control
1 Days	50	40	80	70	95	25
2 Days	20	55	70	100	80	45
8 Days	95	95	100	100	100	5
15 Days	100	100	100	100	100	15
22 Days	100	100	100	100	100	28
29 Days	100	100	100	100	100	85
43 Days	100	35	100	100	100	0
62 Days	50	40	95	100	100	0
128 Days	20	30	95	95	75	5

** Larval mortality percentages calculated after 1 week's exposure to foliage injected with Tameron*

Tameron trial 3: Despite the initial negative results from passive low volume injections, Tameron was taken up and translocated effectively by the tree, albeit slower than the forced injections. Results from passive low volume injections used in phase IV of this research project, similarly showed that Tameron was effectively taken up and translocated, as indicated by bioassay results.

Table 6. *Uraba lugens* mortality (%)* response to three passive low volume injected rates of Tameron

Days post injection	Tameron (10ml)	Tameron (20ml)	Tameron (30ml)	Control
1 Days	5	0	0	0
3 Days	5	5	0	5
71 Days	10	100	80	20
81 Days	10	100	75	5

** Larval mortality percentages calculated after 1 week's exposure to foliage injected with Tameron*

Decis Forte – Deltamethrin

Decis Trial 1: Deltamethrin had minimal effect on either of the two insect species within the time period (24 -48 hours post injection) when, according to the chemical analysis, the concentration should have peaked (Table 7). A third bioassay done 30 days post injection showed higher larval mortality, than the earlier bioassays. Mortality percentages for both these trials are too low to recommend it for effective operational control.

Table 7. *Uraba lugens* and *Paropsis charybdis* mortality (%)* response to stem injected Decis

Days post injection	Uraba control	Uraba	Paropsis control	Paropsis
1 Days	0	0	5	5
2 Days	0	0	10	5
30 Days	10	45	15	20

** Larval mortality percentages calculated after 1 week's exposure to foliage injected with Deltamethrin*

Decis Trial 2: Seven bioassays were conducted on *Uraba lugens* over a three month period post injection (Table 8). Results from the 7 day bioassay should be discarded, as a fungal disease infected the insect population and caused severe (up to 60%) mortality. Similarly the control of the 14 day bioassay had very high larval mortality (75%). However, these insects did not appear to be diseased.
Results from this set of bioassays suggests that Decis was translocated into the foliage of the trees. However, the mortality percentages never reached the threshold level of 80% required for effective larvae control. There was very little consistency and predictability in any of these results.

Table 8. *Uraba lugens* mortality (%)* response to five injected rates of Decis

Days post injection	Decis 10ml	Decis 20ml	Decis 30ml	Decis 40ml	Decis 50ml	Control
1 Days	0	0	5	5	0	0
7 Days**	90	65	50	50	45	85
14 Days***	40	45	35	45	65	75
21 days	0	5	5	5	0	0
47 Days	80	35	60	55	40	20
55 Days	40	20	15	15	60	5
98 Days	5	15	5	15	15	25

**Larval mortality percentages calculated after 1 week's exposure to foliage injected with Deltamethrin*
***Over 60% of larvae died due to a fungal disease*
****Unexplained high mortality in control, no visible fungal disease*

Confidor – Imidacloprid

Confidor Trial 1: It was not possible to detect the imidacloprid by HPLC within 24 or 48 hours post injection. Larval mortality similarly suggested that very little if any translocation of the insecticide had taken place (Table 9).

Table 9. *Uraba lugens* mortality (%)* response to injected Condifor

Days post injection	Confidor 50ml	Confidor 50ml & Water	Control
1 Days	5	0	5
2 Days	0	5	0
17 Days	0	10	0
31 Days	25	25	10

**Larval mortality percentages calculated after 1 week's exposure to foliage injected with imidacloprid*

Confidor Trial 2: Bioassay results 8 days post injection suggested little translocation had taken place, or the injected rates were too low. At 25 days post injection the mortality rates of the highest injected rate, 50ml Confidor Supra, returned the highest mortality rate of 55% (table 10). Unfortunately it was still well below the threshold of 80% mortality. According to available literature, Imidacloprid is the most frequently and popular used insecticide for injection. Therefore it was decided to inject a second set of trees with a higher rate of imidacloprid in the third trial.

Table 10. *Uraba lugens* mortality (%)* response to five injected rates of Confidor Supra

Days post Injection	Confidor 10ml	Confidor 20ml	Confidor 30ml	Confidor 40ml	Confidor 50ml	Control
8 Days	0	0	5	0	0	0
15 Days	**	**	**	**	27	0
25 Days	40	0	10	10	55	10
35 Days	30	5	5	10	10	20
66 Days	10	0	35	5	5	15

** Larval mortality percentages calculated after 1 week's exposure to foliage injected with imidacloprid*
** Not trialed

Confidor Trial 3: Again, there was very little evidence that this second formulation of imidacloprid (EC) had been translocated through the tree, even at high rates (Table 11). The higher rates (90ml/tree) produced a slightly higher mortality, but these mortality rates are very far below the acceptable 80% for recommendation in operational applications.

Table 11. *Uraba lugens* mortality (%)* response to five injected rates of Cofidor Supra

Days post injection	Confidor 60ml	Confidor 70ml	Confidor 80ml	Confidor 90ml	Control
4 Days	0	0	5	27	0
14 Days	10	20	10	20	10
24 Days	0	5	15	20	10
55 Days	5	10	10	15	15

**Larval mortality percentages calculated after 1 week's exposure to foliage injected with imidacloprid*

Calypso - Thiacloprid and Success Naturalyte – Spinosad insecticides
Trial 1: Both insecticides could not be detected via HPLC methods at 1, 2 and 14 days post injection. Neither of these two insecticides managed to achieve the 80% mortality threshold set for successful insect control (Table 12). Subsequent injections in the operational phase produced less than 20% larval mortality.
The active ingredients were extracted, dissolved in water and injected in the operational phase of this project. These injections similarly had almost no effect on insect larvae health.

Table 12. Uraba lugens mortality (%)* response to Calypso and Success insecticide stem injections

Days post injection	Calypso 50ml	Success 50ml	Control
1 Days	0	5	0
2 Days	0	5	0
14 Days	25	50	20
30 Days	25	50	15

**Larval mortality percentages calculated after 1 week's exposure to foliage injected with Thiacloprid & Spinosad*

RECOMMENDATIONS AND CONCLUSIONS

Uraba lugens larvae (the target insect) had no natural resistance to any of the five insecticides tested when applied directly to foliage.
Only Tameron (100%) and Confidor (70%) caused satisfactory mortality to insect larvae after 3 days exposure to the stem uptake bioassays. This was not unexpected because "stem uptake" utilises only the pressure deficit caused by transpiration to actively take the insecticide into the plant tissue. This process is less effective than physical injection into the xylem.

Tamaron – Methamidophos

- Tameron was translocated very quickly (within 24 - 48 hours) 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 4, 5 and 6.
- Persistence in the target foliage was dependant on the injected rate, but effective control was achieved beyond two months after injection.
- Chemical analysis revealed that insecticide concentrations in the foliage declined to almost undetectable levels within 72 hours post injection. Despite these very low concentrations, bioassays conclusively showed that the Tameron was still 100% lethal at 2 months post injection.
- Passive low volume injections of Tameron can be used to control insects on smaller trees.

Decis Forte – Deltamethrin

- There is strong evidence that Decis was translocated into the foliage of Eucalypus trees from a stem injected application.
- Stem injected Decis caused elevated mortality rates of *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 suggested that very little translocation of the active ingredients had taken place. In all four bioassays larval mortality was very low and seldomely higher than the controls.

- A second formulation of imidacloprid was sourced and subsequently trialed in all the remaining Confidor stem injection trials.
- Two separate dose response trials revealed very little proof that imidacloprid was translocated at rates lethal to *Uraba lugens*.
- This formulation (EC) of imidacloprid was also used in operational trials on Eucalyptus globulus at higher rates than those used in trial 3. Again, these results showed no significant effects on larval mortality.

Calypso - Thiacloprid and Success Naturalyte – Spinosad

- Two trials (second in phase III) revealed that both these insecticides had limited efficacy against controlling herbivorous insects.
- The extraction and injection of the active ingredients similarly had minimal effect on larval mortality, causing less than 20% mortality.

ACKNOWLEDGEMENTS

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