

**Evaluation of Systemic Technologies for Introducing Chemical Compounds
into Kiwifruit Vines**

Zespri Project: VI1234

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Executive Summary

Systemic application of antibiotics, elicitors and other compounds into kiwifruit vines is seen as one possible method of combating *Pseudomonas syringae* pv. *actinidae*. This poses the question, "which is the most effective trunk delivery method?"

Trials were conducted on Hort16a in a Psa-free orchard near Opotiki. Spring loaded syringes were compared against stem painting and capsule treatments for translocation of Boric acid and Rubidium chloride into the canopy and fruit at; early bud burst, flowering and full canopy.

Capsules were the most effective method of elevating foliar levels of Rubidium and Boron. Bud burst and flowering were the most opportune times for treatment. Syringes were less effective than capsules and uptake of solutions was slow. Injection of less soluble solutions with syringes would be problematic. Trunk painting was ineffective at all three application times. Fruit was a sink for Rubidium whereas Boron did not enter fruit. Compared against the other two application methods, capsules were easier to apply as well as being effective for getting sap soluble compounds into kiwifruit. A single hole drilled through the centre of the stem can be used repeatedly and shows effective systemic distribution of product. During the course of the trial it was observed that Rubidium is a naturally common constituent of kiwifruit as of course is boron.

Background

The objective of the trial was to compare stem application systems for systemic chemicals. The systemic application of chemicals can be effective against *Pseudomonas syringae* pv. *actinidiae* in kiwifruit¹. Chemicals can be introduced systemically in trees through the direct injection of liquids into the trunk, placement of capsules in xylem tissue or absorption through foliage, trunk or root surfaces. The effectiveness of trunk injection is dependent on injector size and time of application². Systemic application technology can influence plant wounding responses and the subsequent distribution of chemicals from injection sites to other parts of the plant³. This project investigates the effectiveness of three methods of introducing chemicals systemically in kiwifruit vines.

1 Koh et al., (2001 Chemical and cultural control of bacterial blossom blight of kiwifruit caused by *Pseudomonas syringae* in Korea., NZ J. Crop and Horticultural Science, 29: 29-34.

2 Zamora and Escobar, (2001). Injector size and the time of application affects uptake of tree injected solutions. Scientia Horticulturae, 84: 163-177.

3 Doccala et al. (?): Plant Differential wound responses in Southern California Avocado (*Persea americana* cv., Lauraceae) plantations comparing three distinct injection methodologies 2-years following treatment. Arbourjet Internal Report, 17p.

Methodology

The trial was based on 18 yr old Bruno rootstock grafted to Hort16A on an orchard free of Psa and located in Opotiki.

This feasibility study assessed three systemic application technologies plus an untreated control. The treatment solution was based on two tracer chemicals; Rubidium Chloride and Boric Acid.

Treatments:

- 1. Boric acid in gelatin capsules.**
Dose: 3, 1g capsules placed centrally in a 10mm hole drilled through the centre of the stem.
- 2. Rubidium in gelatin capsules.**
Dose: 1, 1g capsule placed centrally in a 10mm hole drilled through the centre of the stem.
- 3. Boric acid applied by plastic syringe (Fig.1).**
20ml of boric solution delivering 3g boron per plant.
A single syringae was applied per plant.
- 4. Rubidium applied by plastic syringe.**
20ml of a rubidium solution (12g in 180 ml/water) giving 1.3g rubidium per plant.
A single syringae was applied per plant.
- 5. Boron applied as a trunk spray.**
3g of boron was dissolved in 200 ml of water to which 1ml of Silwett was added.
This solution was sprayed around the trunk at the base of the plant.
- 6. Rubidium applied as a trunk spray.**
1.3g of Rubidium was dissolved in 200 ml of water to which 1ml of Silwett was added.
This solution was sprayed around the trunk at the base of the plant.
- 7. Control, untreated vines.**



Fig.1. Syringe injecting Rubidium

The above treatments were applied three times:

1. Early bud burst (29/08/11)
2. Flowering (11/10/11)
3. Full canopy (30/11/11)

Leaves and fruit (where appropriate) were picked for residue analysis on 11/10/11 (application 1), 30/11/11 (application 2) and 9/01/12 (application 3) and sent to Hill Laboratories for analysis of boron and rubidium where appropriate.

Trial design: One Ha block, randomized plots.

Replicates: 3 reps of 3 trees = 9 trees per treatment.

Residue analysis: For analysis 3 samples/treatment were analysed. Within each rep samples were taken from each tree and pooled.

RESULTS

1. APPLICATION DATE ONE- Early bud burst (29/08/11)

Treatments were applied at a stage when shoot extension was approximately 50-60cm (Fig.2). The capsule and trunk spray treatments were readily applied. Uptake from syringes was extremely slow and the syringes were removed 24 hours after insertion. When the block was revisited 11/10/11 for further treatment and for collection of leaves for residue analysis (no fruit was formed), symptoms of boron toxicity were observed in leaves from the boron capsule treatment. No boron toxicity was observed in syringe and trunk painting treatments.



Fig 2. State of canopy at time of first treatment (29/08/11)



Fig. 3. State of canopy at time of second treatment (29/08/11)



Fig.4. Boron capsule treatment exhibiting boron phytotoxicity in the leaves



Fig.5. Boron capsule treatment leaves exhibiting yellowing due to boron toxicity

Boron toxicity symptoms observed were mottled yellow and green leaves which were thickened and distorted with necrotic margins.

The residue levels are presented in the following tables and figures.

Table 1: Residue levels of boron and rubidium in leaves of plants treated 29/08/11 and picked 11/10/11. (Hills Report 942908 -13/10/11-21/01/11)

Treatments	Boron (mg/kg)	Rubidium (mg/kg)
Control	4.5 ^b	12.3 ^a
Capsules	14.2 ^a	19.4 ^a
Syringe	5.7 ^b	13.3 ^a
Trunk Spray	4.2 ^b	5.0 ^b

Means separated using Student's t test; values with the same letter do not differ significantly at $\alpha = 0.05$

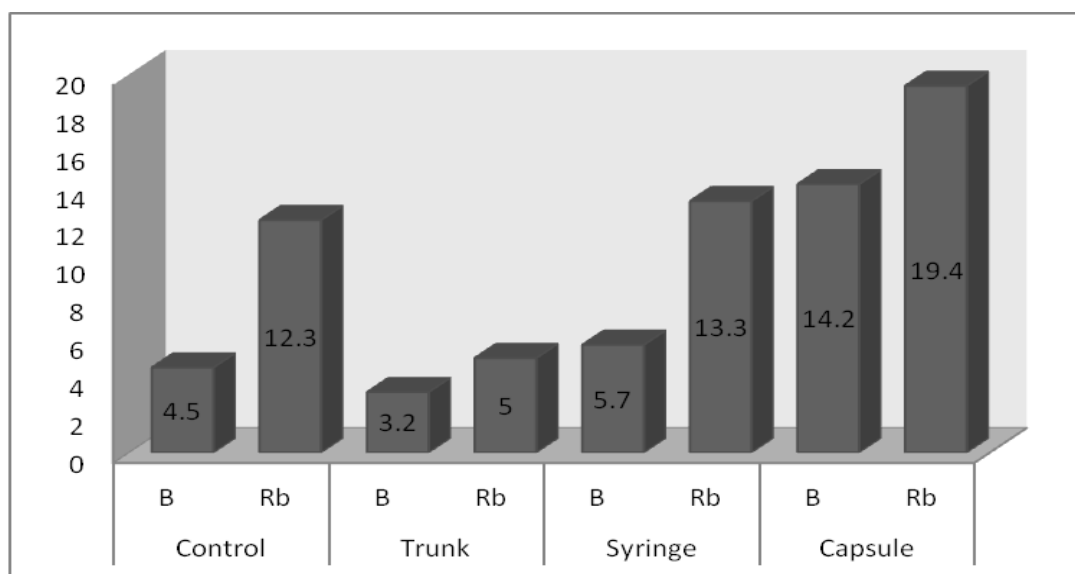


Fig.6. Treatment method v mean levels of boron and rubidium in leaves of plants treated following bud burst

Trunk painting of boron and rubidium was not effective and gave lower foliar levels than the control. The syringe treatment gave slightly elevated levels of boron (>1.2 mg/kg) and rubidium (>1mg/kg). Capsule treatments were the most effective elevating boron by 9.7mg/kg and rubidium by 7.1mg/kg.

We were not expecting to find rubidium in the control vines and were concerned that the rubidium was due to contamination. Accordingly, we picked some some leaves of Hort 16a from South Auckland (22/12/11) and tested them for boron and rubidium at Hill Laboratories. The results gave rubidium levels of 3.1 mg/kg and boron levels of 1.86 mg/kg. Clearly, rubidium occurs naturally in Kiwifruit foliage. (Hills Report 965187 -22/12/11-10/01/12)

2. TREATMENT TWO - Flowering (11/10/11)

Treatments were applied to vines in full canopy with flower buds on 11/10/11 (Fig.7).



Fig. 7. State of canopy when second round of treatments was applied (11/10/11)

Leaves and fruit from this round of treatments were harvested 30/11/11. At this time numerous fruit were present (Fig.8). The third round of treatments was applied to vines at this time. Leaves and fruit from this treatment were harvested for residues on 9/01/12.



Fig. 8. Condition of vines, 30/11/11 when leaves and fruit from the 11/10/11 treatment were collected for assessment of boron and rubidium.

At the collection of leaves and fruit for residues symptoms of boron toxicity were observed in the boron capsule treatment (Fig.9). No other treatments incited boron toxicity. The residue results from fruit and leaves are presented in table 2 and Figs 10 and 11.

For boron (Fig.10) there was no movement of boron into the fruit regardless of application method suggesting that fruit was not a sink for boron. For the foliage the syringe and trunk treatments did not elevate boron levels above the control level. The capsule treatment elevated boron levels by 57%. This was confirmed by the observation of boron phytotoxicity in this treatment (Fig 9).

For rubidium (Fig.11) trunk and capsule treatments failed to elevate rubidium levels above control fruit. The syringe treatment showed a slight elevation of rubidium (0.5 mg/kg) over the control. As with boron, the fruit are not a strong sink for rubidium. For the foliage the syringe and trunk treatments elevated rubidium levels by 1.2/1.4 mg/kg above the control level. The syringe treatment elevated boron levels by 64%.



Fig.9. Boron toxicity in leaves from boron capsule treatment

Table 2: Residue levels of boron and rubidium in leaves of plants treated 11/10/11 and picked 30/11/11. (Hills Report No 958843/ 2/12/11-13/12/11)

Treatments	Boron (mg/kg)	Rubidium (mg/kg)
Control	7.5 ^{ab}	3.8 ^b
Capsules	17.3 ^a	5.2 ^b
Syringe	6.3 ^b	10.7 ^a
Trunk Spray	7.2 ^{ab}	5.0 ^b

Means separated using Student's t test; values with the same letter do not differ significantly at $\alpha = 0.05$

Table 3: Residue levels of boron and rubidium in fruit of plants treated 11/10/11 and picked 30/11/11.
(Hills Report No 958843/ 2/12/11-13/12/11)

Treatments	Boron (mg/kg)	Rubidium (mg/kg)
Control	1.9 ^{ab}	2.4 ^a
Capsules	1.5 ^b	2.2 ^a
Syringe	1.6 ^b	2.9 ^a
Trunk Spray	1.9 ^a	2.1 ^a

Means separated using Student's t test; values with the same letter do not differ significantly at $\alpha = 0.05$

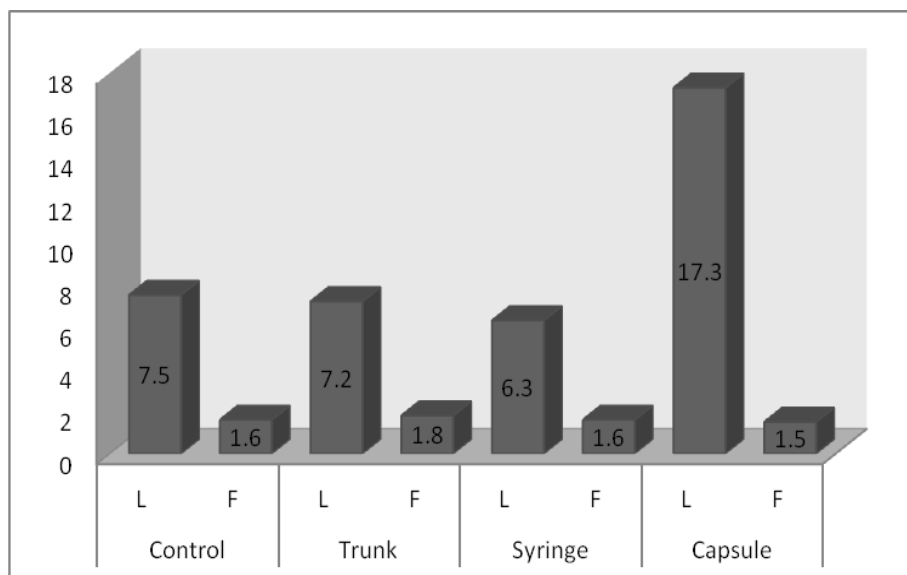


Fig.10. Treatment method v mean levels of boron in leaves and fruit of plants treated at flowering (11/10/11)

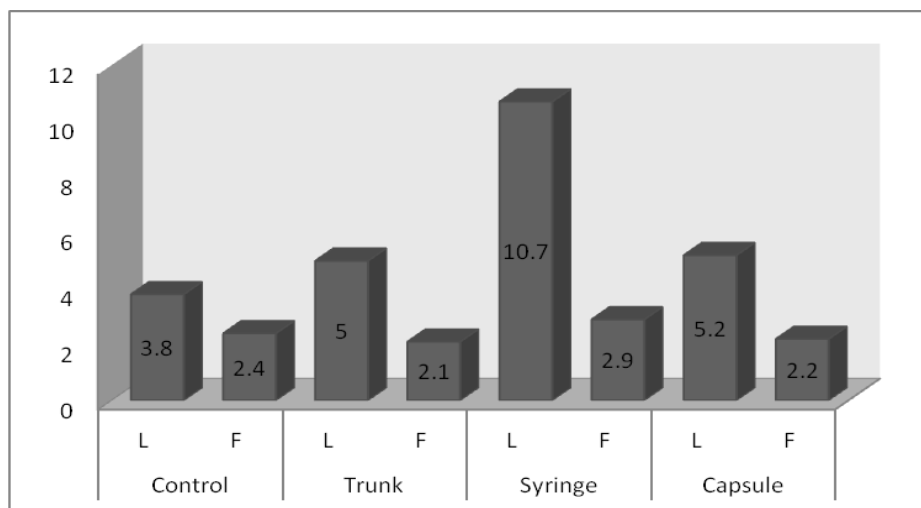


Fig.11. Treatment method v mean levels of rubidium in leaves and fruit of plants treated at flowering (11/10/11)

The full canopy with fruit treatment applied 30/11/11 was harvested 9/01/12. At this stage the canopy was loaded with fruit Fig 12.



Fig.12. State of canopy at collection of fruit and leaves for residue analysis 9/01/12

The residue results are presented in Table 4 - 5 and Figs 12 and 13. At the collection of leaves and fruit for residues no symptoms of boron toxicity were observed in the boron capsule treatment.

For boron (Fig.12) there was no movement of boron into the fruit with trunk spraying. The syringae treatment elevated the boron level by 1 mg/kg, the capsule treatment by 0.3 mg/kg. These differences are minor and as with previous applications suggest that fruit is not a sink for boron. For the foliage the trunk treatment did not elevate boron levels above the control level. The capsule treatment elevated boron levels by 21%, the syringe method by 7%. (Fig 12).

For rubidium (Fig.13), the trunk treatment failed to elevate rubidium levels above control fruit. The syringe treatment showed a slight elevation of rubidium (1.0 mg/kg) over the control. The capsule treatment elevated fruit levels by 4.4 mg/kg (262%). Application of rubidium to vines with a full canopy (via capsules) moved rubidium into the fruit. As with boron fruit have low levels of rubidium and are not a strong sink for this element.

For the foliage only capsules elevated rubidium (+14%) above the control level. The syringe and trunk treatments exhibited lower rubidium levels than the control.

Table 4: Residue levels of boron and rubidium in leaves of plants treated 30/11/11 and picked 9/01/12.
(Hills Report No 967636/ 11/01/12-17/01/12)

Treatments	Boron (mg/kg)	Rubidium (mg/kg)
Control	5.6 ^{ab}	3.8 ^b
Capsules	6.8 ^a	6.4 ^a
Syringe	6.0 ^{ab}	3.7 ^b
Trunk Spray	4.5 ^b	4.3 ^b

Means separated using Student's t test; values with the same letter do not differ significantly at $\alpha = 0.05$

Table 5: Residue levels of boron and rubidium in fruit of plants treated 30/11/11 and picked 9/01/12.
(Hills Report No 967636/ 11/01/12-17/01/12)

Treatments	Boron (mg/kg)	Rubidium (mg/kg)
Control	1.5 ^{ab}	2.7 ^b
Capsules	1.8 ^a	7.1 ^a
Syringe	1.6 ^{ab}	3.9 ^b
Trunk Spray	1.4 ^b	2.8 ^b

Means separated using Student's t test; values with the same letter do not differ significantly at $\alpha = 0.05$

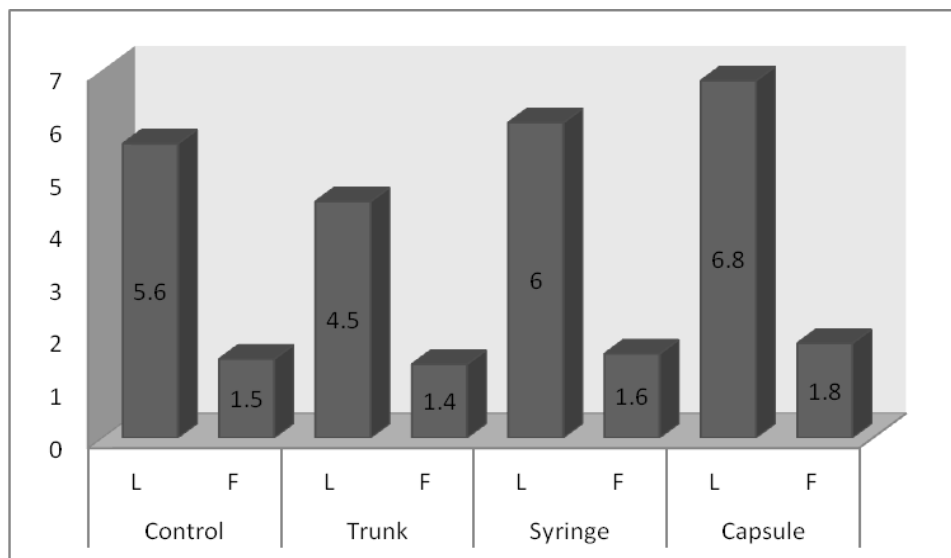


Fig.13. Treatment method v mean levels of boron in leaves and fruit of plants treated at full canopy (30/11/11)

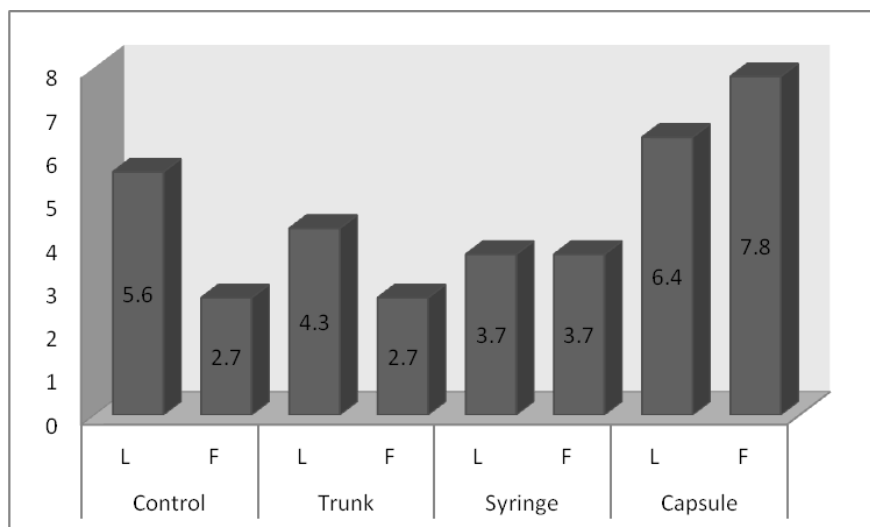


Fig.14. Treatment method v mean levels of rubidium in leaves and fruit of plants treated at full canopy (30/11/11)

Optimum Treatment Method

The mean levels of boron in the leaves over the three inoculation times are presented in Fig 15. The data shows that the capsule method was the most effective technique for elevating levels of boron and rubidium in foliage of Hort 16a. Trunk application was ineffective as was the syringe for boron. The syringe method was more effective for rubidium (which is highly mobile), than for boron.

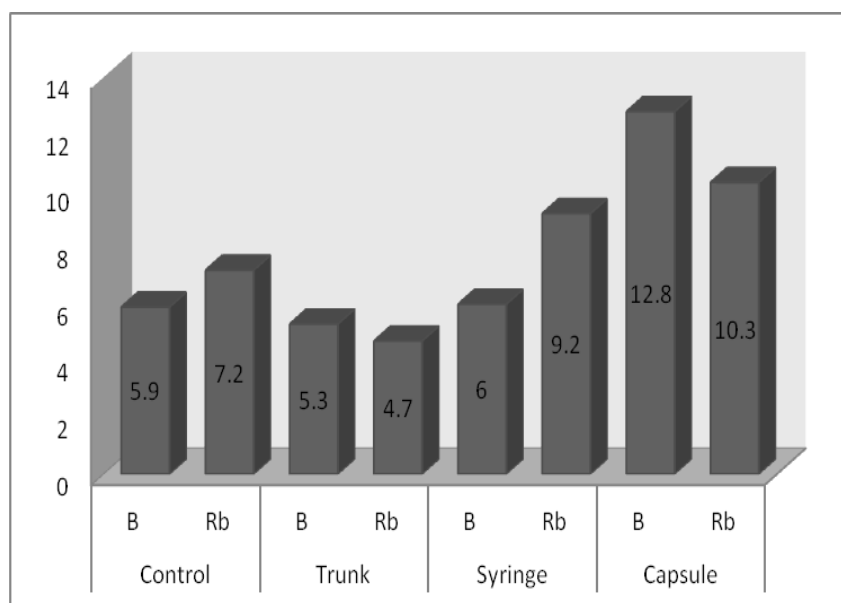


Fig 15 Summary of mean boron and rubidium levels in leaves over three application times

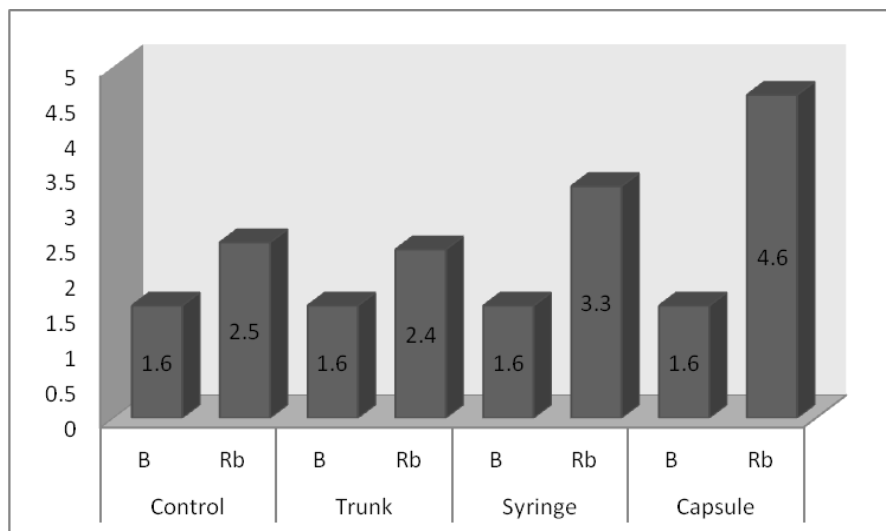


Fig 16 Summary of mean boron and rubidium levels in fruit over two application times

The mean levels of boron in the fruit over the three inoculation times are presented in Fig 16. The data shows that fruit were not a sink for boron regardless of treatment method. Rubidium was more mobile and did move into fruit. The capsule technique was more effective than the syringe method as found previously for leaves. Trunk application again was ineffective.

Optimum Time for Treatment

Previous studies have shown that capsules are the most effective method for getting boron and rubidium into foliage of Hort 16A. Using this method the most effective times for boron treatment were bud break and flowering. Treatment at full canopy was the least effective time (Fig 17). Treatment with rubidium at flowering gave the highest levels in the canopy (Fig 17).

Fruit were not a strong sink for boron neither at flowering nor at full canopy (Figs 10 and 13). Rubidium entered fruit more strongly at full canopy than at flowering (Figs 11 and 14).

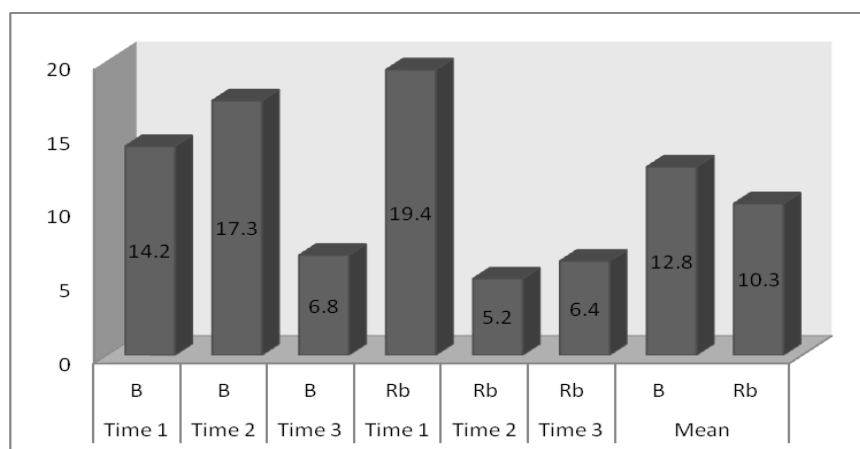


Fig 17. Effect of treatment time with capsules on resultant boron and rubidium levels in foliage.
 At right - mean levels in control foliage over three treatment times.

Discussions

The results of this trial have shown that treatment of Hort 16A with capsules is the most effective way to elevate foliar levels of boron and rubidium. Bud burst and flowering were the most opportune times for treatment. Trunk spraying with boron and rubidium was comparatively ineffective. Notwithstanding, different compounds may behave differently as was observed between boron and rubidium.

Boron and rubidium are highly soluble and very mobile and along with phosphorous acid are highly suitable for syringe injection. Injection of less soluble compounds would be problematic.

The syringe injection method was inefficient and slow from a practical application point of view, requiring a follow up visit the next day to remove syringes. This was a major problem at early bud burst and even during flowering and at full canopy when not all of the syringes had drained.

Capsules were easy to use, were rapid to apply and rely on plant xylem fluid for dissolving the contents into solution.

This is ideal for compounds which are less soluble in water as xylem fluid has a strong ability to dissolve compounds. The presence of boron phytotoxicity in foliage further demonstrates the effectiveness of this treatment regime.

Conclusions

It should be possible to formulate an effective stem tablet /capsule treatment to protect kiwifruit grafts from Psa during early establishment. From our field observations Hort16a is too susceptible to protect from Psa via stem-administered treatments especially since foliar sprays have not been effective.

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To Zespri and all the grower suppliers whom have directly and indirectly funded this work, Omnia trust that the findings will go some way to giving an insight to the mechanics of kiwifruit vines and the potential future directions for Systemic control of Psa in the future

Finally, greatly appreciated was the guidance and support from Zespri.