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Final Report

Fungicide Trials Associated with Myrtle Rust Control in New Zealand

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Myrtle rust infection on *Lophomyrtus* x*ralphii* 'Black Stallion'

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Final Report: Fungicide Trials Associated with Myrtle Rust Control in New Zealand

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More information

Cuddy, W.S., Carnegie, A., Biosecurity and Food Safety, Elizabeth Macarthur Agricultural Institute, Menangle NSW 2568

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Contents

Executive Summary

Myrtle rust is a fungal disease of members of the Myrtaceae plant family, It was detected in New South Wales in 2010 and in New Zealand in 2017. This study investigated the role of different fungicides and different timings of application relative to a single inoculation time for protectant and curative activity against myrtle rust. Of the chemical options investigated Amistar Xtra, Scorpio and Bayfidan were generally the best options for protection and control of myrtle rust infection in one variety of *Metrosideros* and one variety of *Lophomyrtus*. Future work should consider how to improve the coverage of plant canopies and stems for better chemical control of myrtle rust.

Introduction

Myrtle rust is a disease of plants from the Myrtaceae, caused by the obligate biotrophic fungal pathogen *Austropuccinia psidii*. Myrtle rust was detected in New South Wales in 2010 and it has since spread widely up the temperate and tropical eastern coast of Australia. In 2017 myrtle rust was detected in New Zealand. This trial was requested by the New Zealand Ministry for Primary Industries to start exploring options for the chemical control of myrtle rust to aid in eradication and management programs for the country.

Materials and Methods

Plant and rust materials

Two plant varieties were used in the trial: *Metrosideros excelsa* 'nana' and *Lophomyrtus* x *ralphii* 'Black Stallion'. The *Metrosideros* were in 275 × 140 mm pots whereas the *Lophomyrtus* were 425 × 40 mm tubestock. Plants were separated by genus and grown in the completely randomised design provided by NZ MPI which included 5 replicate plants per treatment. Plants were maintained in shadehouse facilities at the Elizabeth Macarthur Agricultural Institute prior to inoculation.

The rust urediniospores used in the trial were the standard working culture of myrtle rust (=622) used at the Plant Breeding Institute at the University of Sydney. At inoculation, rust urediniospores were suspended in IsoparL mineral oil and applied using an Iwata air brush and air compressor at 25 psi. Water agar plates were included with the plants at inoculation and counts determined there to be an average of 325 urediniospores cm^{-2} applied to the plants in the trial. After inoculation plants were incubated for 36 hours in the dark at 20°C and 100% humidity provided by ultrasonic humidifiers. After incubation, plants were transferred to the greenhouses at the Elizabeth Macarthur Agricultural Institute, Menangle and grown maintained at 22±2°C under ambient light conditions.

Trial schedule

The general schedule of the trial is summarised in Table 1.

Table 1. Schedule of inoculation, fungicide applications and notes taken.

Date	Trial step	Spray code
23/11/2017	14 days pre-inoculation fungicide application	pre_14
30/11/2017	7 days pre-inoculation fungicide application	pre_7
06/11/2017	Inoculation and incubation	
08/11/2017	Plants removed from incubation	
13/11/2017	7 days post inoculation fungicide application	post_7
17/11/2021	First pustules noticed on plants	
20/11/2017	14 days post inoculation fungicide application	post_14
21/11/2017	*Metrosideros* trial scored	
23/11/2017	17 days post inoculation fungicide application	post_7
24/11/2017	*Lophomyrtus* trial scored	
27/11/2017	21 days post inoculation fungicide application	post_21
30/11/2017	24 days post inoculation fungicide application	post_14
06/12/2017	Both trials washed to remove rust from leaves and stems	
11/12/2017	Final scoring of both trials for curative activity of fungicides	

Fungicide applications

Fungicides were mixed in 500 mL volumes with town water and applied in a Generation III Research Spray booth (Devries Manufacturing) calibrated to apply 500 L ha^{-1}. A Teejet DG11002 flat fan nozzle was used to apply treatments at 50 cm above canopy height. The fungicides applied are shown in Table 2. As a general rule, fungicides were applied at the label rate (rate 1) and twice the label rate (rate 2). Fungicides were applied at: 14 days pre inoculation (pre_14); 7 days pre inoculation (pre_7); 7 and 17 days post inoculation (post_7); 14 and 24 days post inoculation (post_14); and at 21 days post inoculation (post_21).

Disease assessments

The *Metrosideros* trial was scored 15 days after inoculation. For this trial the leaf area affected (LAA) was scored on 3 randomly selected leaf pairs that were fully emerged at the time of inoculation to give a total of 6 LAA measures per pot. These values were averaged to give the representative LAA per pot. At 35 days after inoculation the LAA was again assessed. At this second scoring, the LAA was determined by scoring only the percentage leaf area affected by actively sporulating pustules for the pair of leaves that were fully emerged on the dominant stem at the time of inoculation. Both the abaxial and adaxial sides of the leaf were scored.

The *Lophomyrtus* trial was scored 18 days after inoculation. For this trial the LAA was scored on both leaves of the highest leaf pair that was fully emerged at the time of inoculation and averaged to give the LAA per pot. The internode below the fully emerged leaves was also scored to indicate the internode area affected (IAA). Due to severe leaf necrosis caused by

myrtle rust infections in *Lophomyrtus* plants during the trial, the second scoring on day 35 after inoculation only scored the actively sporulating pustules on the same internode. For this second scoring, the internode was scored from 1-4 where: 1 was clean (i.e. no infection); 2 was a completely killed infection (indicated by hyaline pustules); 3 indicated an infection that was >50% killed but with some actively sporulating pustules still on stems; and 4 indicated actively sporulating pustules on the internode region with no obvious interruption to infection from treatments.

Table 2. Fungicides and rates used in the trial

Fungicide	Active ingredient(s)		FRAC Group	Manufacturer	Rate 1 (mL/g per 500 mL solution)	Rate 2 (mL/g per 500 mL solution)
Scorpio	Tebuconazole Trifloxystrobin	200 g/L 100 g/L	3 11	Bayer	0.375 mL	0.75 mL
Amistar Xtra	Cyproconazole Azoxystrobin	80 g/L 200 g/L	3 11	Syngenta	0.5 mL	1 mL
Aliette WG	Fosetyl Aluminium	800 g/kg	33	Bayer	1.25 g	2.5 g
Saprol	Triforine	190 g/L	3	SIPCAM	0.5 mL	1 mL
Bayfidan 250 EC	Triadimenol	250 g/L	3	Bayer	0.25 mL	0.5 mL
Plantvax 750 WP	Oxycarboxin	750 g/kg	7	Arysta	0.65 g	1.3 g
Nordox 750 WG	Copper (Cuprous oxide)	750 g/kg	M1	Nordox	0.5 g	0.75 g
Folicur 430 SC	Tebuconazole	430 g/L	3	Bayer	0.15 mL	0.3 mL

Statistical analyses

Statistics presented in this report were prepared using RStudio version 1.1.383 and R version 3.4.3 for Windows. Treatments are presented as TREATMENT_RATE_TIMING, e.g. Scorpio_1_pre_7 is the treatment in the design using Scorpio at rate 1 applied 7 days pre inoculation. ANOVA was used to compare the treatments to the water controls for each spray timing. In cases where ANOVA indicated significant differences between treatments, a Tukeys

pairwise comparison was used to compare all pairs of treatments using the MULTCOMP package within R. Due to a handling error several samples lost their labels from the *Lophomyrtus* trial. To enable a balanced analysis, one replicate was randomly chosen for each treatment that did not have missing labels so a total of four replicates could be analysed for each treatment.

Results

First pustules erupted 11 days post inoculation. At 21 days post inoculation leaf distortion in both *Metrosideros* and *Lophomyrtus* plants was evident. In the *Lophomyrtus* plants, leaf necrosis and stem distortion was also becoming pronounced.

The average LAA for in water-treated samples was only 16% for *Lophomyrtus* and 12% for *Metrosideros*. Average IAA in water-treated *Lophomyrtus* plants was 26% indicating that the stems of *Lophomyrtus* may be more susceptible than leaves to infection by myrtle rust. The results from the ANOVA are summarised in Table 3. For *Metrosideros*, only the protectant application of fungicides 7 days prior to inoculation significantly reduced the LAA. Results are summarised in Figure 1. Tukey's multiple comparisons showed that both rates of Amistar Xtra and Scorpio, and the higher rate of Bayfidan significantly reduced LAA by myrtle rust relative to both the water and Fosetyl Aluminium treatements. In the case of Fosetyl Aluminium, plants treated with the lower rate of Bayfidan also had significantly reduced LAA.

Curative effects were significant on both the adaxial and abaxial leaf surfaces when applied at 7 and 17 (post_7); and 21 days post inoculation. Application of fungicides at 14 and 24 days post inoculation (post_14) significantly reduced the sporulating leaf area on the abaxial leaf surface. For the post_7 treatments, both rates of Amistar Xtra, Bayfidan, Saprol and Scorpio had significantly reduced LAA on adaxial leaf surfaces. In all cases except the lower dose rate of Bayfidan, the same treatments had significantly lower adaxial LAA than plants treated with Fosetyl Aluminium. On the abaxial leaf surfaces, the only significant difference was between the lower rate of Amistar Xtra and the higher rate of Fosetyl Aluminium. Results are summarised in Figure 2. For the post_14 abaxial results, Tukey's multiple comparisons did not show any significant differences between any pairwise comparisons. A single fungicide application 21 days post inoculation did have a significant effect at both rates of Amistar Xtra, Bayfidan, Scorpio and the lower rate of Saprol on the adaxial leaf surface. On the abaxial leaf surface, both rates of Amistar Xtra, Saprol, Scorpio and Fosetyl Aluminium and the lower rate of Bayfidan reduced leaf area affected by actively sporulating rust. These results are summarised in Figure 3.

Table 3. *P* values from Analysis of Variance for fungicide treatments. *P* values in bold type indicate significant variances.

		pre_14	pre_7	post_7	post_14	post_21
Metrosideros	Leaf area affected	0.294	**<0.001**	0.068	0.821	0.878
	Adaxial LAA curative			**<0.001**	0.051	**<0.001**
	Abaxial LAA curative			**0.001**	**0.029**	**<0.001**
Lophomyrtus	Leaf area affected	0.143	**0.001**	**0.002**	0.414	0.778
	Internode area affected	0.291	**0.017**	0.09	0.501	0.303

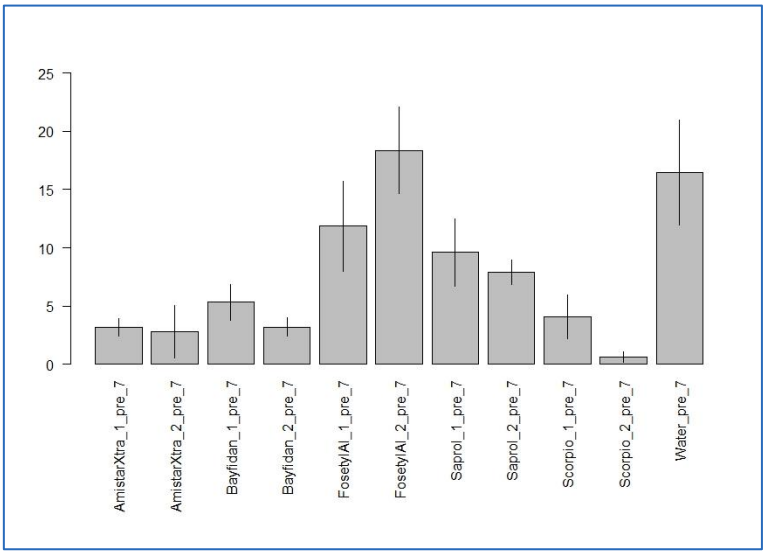
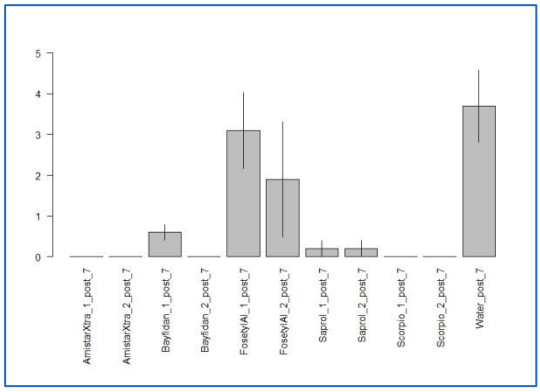


Figure 1. Summary graph showing the effect of applying fungicides 7 days prior to inoculation on the leaf area affected (LAA) of *Metrosideros excelsa*. Bars indicate standard error.

(a)



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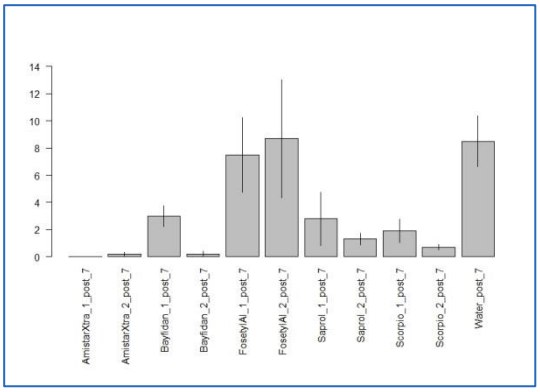
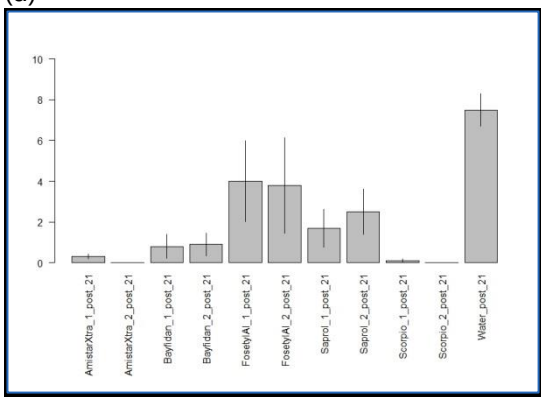


Figure 2. Summary graphs showing the effect of applying fungicides 7 and 17 days post inoculation on the (a) adaxial and (b) abaxial leaf area affected (LAA) of *Metrosideros excelsa*. Bars indicate standard error.

(a)



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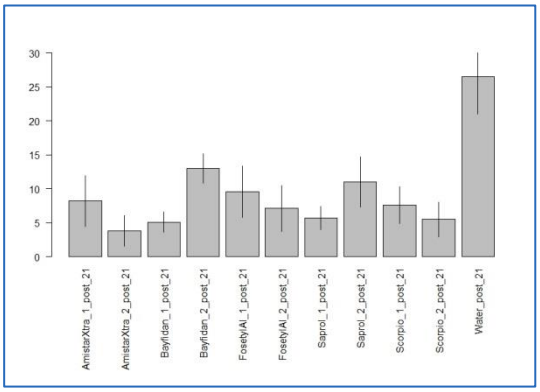

Figure 3. Summary graphs showing the effect of applying fungicides 21 days post inoculation on the (a) adaxial and (b) abaxial leaf area affected (LAA) of *Metrosideros excelsa*. Bars indicate standard error.

In *Lophomyrtus*, only applying fungicide treatments at 7 days prior to inoculation (pre_7) and 7 and 17 days post inoculation (post_7) had a significant effect on the leaf area affected (LAA). Only the pre_7 treatments had a significant effect on internode area affected (IAA). For pre_7 both rates of Folicur and Scorpio had lower LAA than plants treated with Fosetyl Aluminium (Figure 4). Tukey's multiple comparisons did not show any significant pairwise differences between treatments on IAA at pre_7. For the post_7 treatments, no significant differences to treatment with water were observed, though the high rate of Amistar Xtra and the lower rate of Scorpio both significantly reduced LAA relative to the higher rate of Nordox.

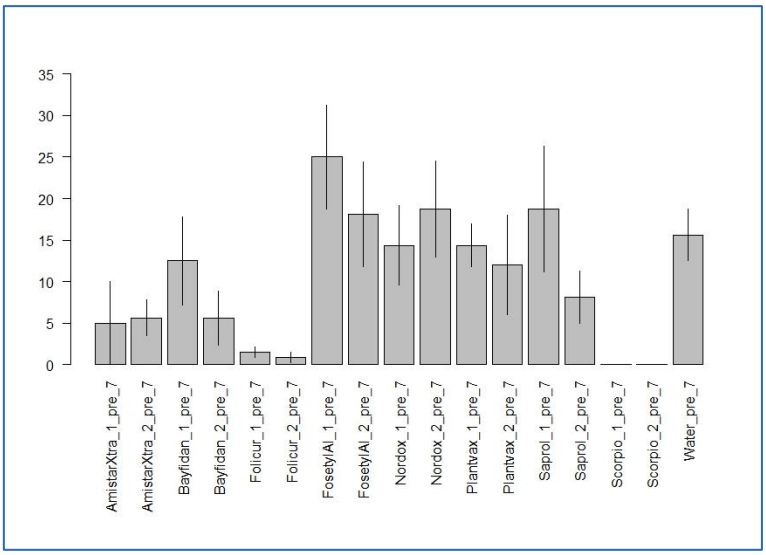

Figure 4. Summary graph showing the effect of applying fungicides 7 days prior to inoculation on the leaf area affected (LAA) of *Lophomyrtus* x *ralphii*. Bars indicate standard error.

The curative activity of all fungicide treatments was scored on *Lophomyrtus* internodes at 35 days post inoculation (Figure 5). Due to the categorical nature of the scoring no statistical analysis was conducted. General trends can be observed, namely that applications either 7 days prior or 7 and 17 days post inoculation provided the best protection for stems. Generally Amistar Xtra, Scorpio and Bayfidan provided the best protection for internodes and Fosetyl Aluminium, Plantvax, Saprol and Nordox provided the least protection or curative activity to internodes.

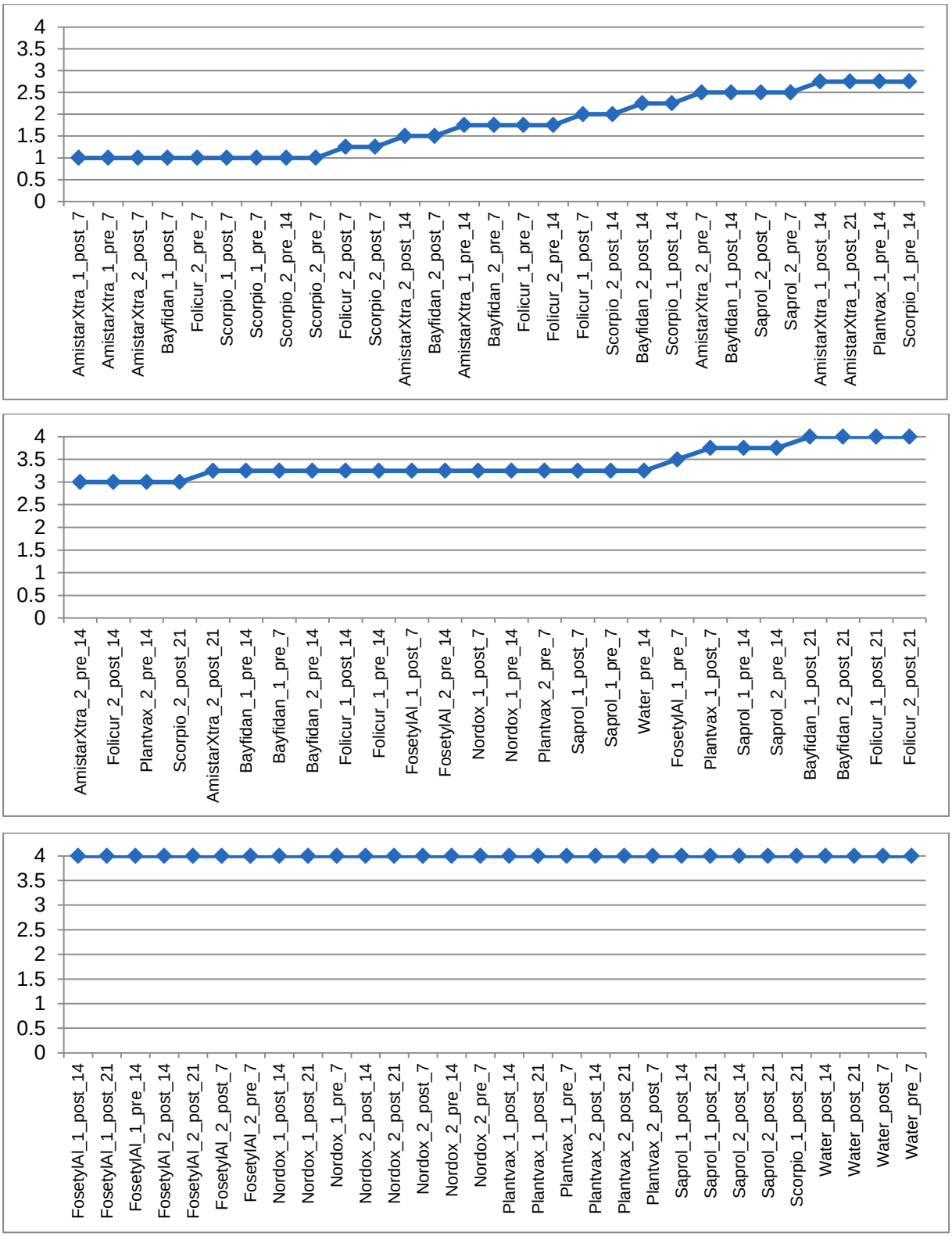

Figure 5. Graph showing the average score of internode infection with myrtle rust on *Lophomyrtus* x *ralphii* plants. Treatments are organised from lowest to highest average category. On the y axis, 1 indicates clean internode (i.e. no infection); 2 was a completely killed infection (indicated by hyaline pustules); 3 indicated an infection that was >50% killed but with some actively sporulating pustules still on stems; and 4 indicated actively sporulating pustules on the internode region with no obvious interruption to infection from treatments.

Discussion and Conclusion

Across the trial Amistar Xtra, Scorpio and Bayfidan appear to be the most promising options for controlling myrtle rust in *Metrosideros* and *Lophomyrtus*. The results presented indicate that Fosetyl Aluminium, Nordox and Plantvax are not likely to be valuable protectants or curative products for myrtle rust.

Inoculation density across the trial was patchy. Despite an average of 375 urediniospores of myrtle rust being applied per cm^2 it was common to find one leaf heavily infected while its paired leaf was immune. Part of the problem is likely the incubation. It is recommended that next time the incubation be limited to 24 hours and for more spacing to be included between plants to ensure good penetration of humidity from the ultrasonic humidifier. It is also recommended that next time a trial of this sort with these plants, and especially members of the genus *Metrosideros* are used, that all plants are pruned one month prior to the first fungicide application to get new flush coming through at a more consistent rate.

The *Metrosideros* plants generally grew a new pair of leaves every week, so while the 14 day pre-inoculation spray provided protection for the leaves scored there was significant infection on younger leaves. Similarly, new growth was often severely infected on *Lophomyrtus* plants though they grew leaves more slowly. As such, results from the pre_7 and post_7 treatment times provided the most reliable indication of which treatments are likely to provide the best protectant and curative activity for myrtle rust.

The variation in water results across treatment times (data not shown), and particularly in relation to the 21 day post-inoculation spray, indicates that there has likely been some vapour effects on rust sporulation between treatments during transport between the Plant Breeding Institute at the University of Sydney and the Elizabeth Macarthur Agricultural Institute. One solution to this problem would be to limit trials to tubestock in which case they can use the space at the Plant Breeding Institute at the University of Sydney in which case they may need to be contracted via the University rather than the NSW Department of Primary Industries.

Generally, controlling myrtle rust on *Lophomyrtus* stems and the abaxial leaf surface of *Metrosideros* was more difficult. Part of the problem is ensuring spray coverage of stems which have a low exposure area to a flat fan nozzle and the abaxial leaf surface which rarely gets effective fungicide application from a vertical fungicide spray. It was not uncommon in this trial to find treatments that effectively controlled myrtle rust on the adaxial, and so visible, leaf surface of *Metrosideros excelsa* while the abaxial leaf surface was still heavily infected. Results observed from the post_7 sprays may indicate that the route the fungus uses to colonise the plants may provide aid in fungicide penetration of the leaf material through chemical translocation through the fungal hyphae that are the infection units. This hypothesis requires validation through independent studies that examined the infection process of myrtle rust in conjunction with chemical applications.