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Tiakitanga Pūtaiao Aotearoa

MPI 18607 Project Report

Pilot trials for control of myrtle rust using fungicides

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Executive summary

Background
This research is the first step towards identifying fungicides suitable for myrtle rust control in New Zealand. Our approach was to:

1. Identify an adjuvant that would provide the best spread of the fungicides selected as potentially effective against myrtle rust. Spreading is an important component of efficacy often overlooked in disease management studies.
2. Develop methods for artificially infecting New Zealand Myrtaceae under highly controlled conditions. This was important for us to be able to conduct efficacy tests under the containment conditions (PC1 Micro-organisms) applied to working with this pathogen.
3. Test the preventive potential of three fungicides (Timorex Gold, Vandia and Radial) applied with adjuvant at a 'recommended dose' to control myrtle rust on on *Metrosideros excelsa* (pōhutukawa).

Key results

- The adjuvant Actiwett, an alcohol ethoxylate, provided maximum spread of seven products tested on both the upper and lower surfaces of *M. excelsa* leaves.
- For the first time in New Zealand, we achieved successful infection of *M. excelsa* and *Lophomyrtus bullata* (ramarama) in containment by artificial inoculation.
- We developed a novel spray application technique that used turbulence to ensure maximum fungicide deposition and coverage on *M. excelsa* leaves in controlled conditions.
- Of the three fungicides tested for preventative control, Radial was the most effective.The natural product extract or "biological", Timorex Gold, was not effective.

Implications of results for the client
This research was collaborative and brought together scientists from Scion, MPI and Plant Protection Chemistry with expertise in pest management, plant pathology and spray technology. The effectiveness of Actiwett was an important outcome for all further testing of fungicide products on *M. excelsa*. We can now focus on identifying suitable fungicides. The spray application technique will enable us to better simulate deposition of fungicides delivered via aerial platforms.
The study is an initial step towards developing short term management options for myrtle rust control on Myrtaceae using fungicides. However, it is by no means enough to develop recommendations for a control strategy.

Further work
Further work to consolidate these results and expand our understanding of factors which optimise efficacy needs to be conducted before any recommendations on operational control can be made. Because there is no current operational myrtle rust control approach, further research is a high priority.We recommend that:

1. A comprehensive survey is conducted with Māori, stakeholders, end-users and land-owners to determine their need for management tools and requirements to support a license to operate, particularly where there is cultural sensitivity around potential valuable tools.
2. Depending on outcome from above consultation, that appropriate steps are taken to determine how to make fungicides and application tools acceptable by end-users.
3. Further trials testing other fungicides known be effective against myrtle rust on species other than *M. excelsa* are carried out. The preventative and curative properties of these fungicides would need to be identified as well as their relative persistence on plant surfaces.
4. Dose response trials are conducted to identify the optimum dose at which to apply fungicide. Further, factors affecting spread, retention and uptake of key fungicides need to be investigated to optimize the efficacy of the most effective fungicides.
5. Field trials be conducted to determine the best application platform and method in different scenarios (isolated trees, small woodlots or dispersed individuals).
6. Because teliospores were for the first time identified on infected leaves of treated plants, trials are needed to determine the effect of fungicides on the different life cycle stages of *A. psidii*.
7. The environmental impacts of key fungicides be evaluated.

Contents

1 Project background

To better understand myrtle rust and limit its impact in New Zealand, the Ministry for Primary Industries commissioned a comprehensive research programme in 2017 with more than 20 projects valued at over $3.7 million. Projects in this programme were completed by June 2019.

The projects covered research in the following themes:

- Theme 1 - Understanding the pathogen, hosts, and environmental influence.
- Theme 2 – Building engagement and social licence: Improved understanding of public perceptions and behaviours to allow better decisions about investment, improved design of pathway control strategies and maintain social license for use of management tools.
- Theme 3 – Te Ao Māori: Greater understanding of Te Ao Māori implications of myrtle rust in order to support more effective investments, and improved use of Mātauranga, specific Māori knowledge, and kaupapa Māori approaches in management regimes.
- Theme 4 – Improving management tools and approaches: Improved diagnostic and surveillance speed, accuracy and cost-effectiveness, supporting eradication efforts and enabling scaling up of surveillance efforts for a given resource. More effective treatment toolkits to avoid emergences of MR resistance to treatments and to enable disease control over increasingly large scales that will lead to reduced or avoided impacts.
- Theme 5 - Evaluating impacts and responses: Improved understanding of environmental, economic, social and cultural, impacts to inform risk assessment and management and to communicate implications to decision/makers and stakeholders.

This report is part of the MPI commissioned research under contract MPI18607 which addressed research questions within Theme 2, 4 and 5.

Text in the report may refer to other research programmes carried out under the respective theme titles.

2 Introduction

Fungicides are a significant component of many effective disease management strategies. Since the incursion of myrtle rust, a disease caused by the pathogen *Austropuccinia psidii*, in New Zealand in May 2017 (Beresford et al. 2018), the only interim operational chemical control method has been that recommended by MPI in New Zealand. These recommendations were based on broad generic information on efficacy in relation to rust control and are not specific for New Zealand host plants. About 29 native Myrtaceae species (six genera) are identified in New Zealand in addition to a large number of exotic Myrtaceae (Buys et al. 2016). Most of these Myrtaceae including the iconic pōhutukawa (*Metrosideros excelsa*), mānuka (*Leptospermum scoparium*) and ramarama (*Lophomyrtus bullata*) are susceptible to myrtle rust. Myrtle rust is already an established threat to most of New Zealand's indigenous ecosystems and plant industries (such as mānuka honey and oil, forestry, horticulture, plant propagation and cut flowers).

Presently in New Zealand, apart from what has been occurring operationally in the national incursion response, there are no fungicides tested for efficacy against this disease on susceptible Myrtaceae species.. However, based on a comprehensive literature review conducted in 2018 (Chang et al. 2018), together with outcomes from controlled, artificial inoculation trials conducted in Australia (Pathin et al. submitted) some promising fungicides were identified and recommended for further testing.

The research presented in this report represents the first steps towards evaluation of suitable fungicides for control of myrtle rust in New Zealand. Based on the outcome of previous work (Chang et al. 2018 and Pathin et al. submitted), our approach was to:

1. Identify an adjuvant that would provide the best spread of the fungicides selected as potentially effective against myrtle rust. Spreading is an important component of efficacy often overlooked in disease management studies.
2. Develop for the first time in New Zealand methods of artificially infecting iconic Myrtaceae under highly controlled conditions. This was important for us to be able to conduct efficacy tests under the containment conditions (PC1 Micro-organisms) applied to working with this pathogen.
3. Test the preventive potential of three fungicides applied with adjuvant at a 'recommended dose'. The focus of the preventative trial was on *M. excelsa* due to limited access to other native species in New Zealand.

Due to the restrictions on working with the myrtle rust pathogen in an open-environment all of our research was conducted in a PC1 Micro-organism Containment Facility at Scion. Furthermore, these restrictions meant that we could only conduct trials that tested the preventative activity of the fungicides, as movement of infected material was not permissible or practically feasible.

3 Materials and methods

3.1 Adjuvants selection trial

The aim of the adjuvant selection trial was to determine the effect of different classes of adjuvant on spreading of selected products. Three adjuvant classes were tested across seven products with different active ingredients. This research was conducted by Scion in collaboration with Plant Protection Chemistry New Zealand (PPCNZ).

Three healthy *M. excelsa* (pōhutukawa) trees were selected in the Rotorua environs, all at separate locations. Multiple fresh shoots bearing the youngest healthy foliage (i.e. leaves attached to stems were harvested) were sampled from each tree in the early morning. These were transported to PPCNZ with stems kept moist and maintained at 20 ºC. Treatments were applied to adaxial and abaxial surfaces of the youngest leaves on each shoot (Fig.1). The surfactants tested were chosen to represent typical adjuvant classes which are used in fungicide sprays. The adjuvants used in the study were; Actiwett® (a non-ionic, 950 g L^{-1} linear alcohol ethoxylate, Batch #148504, Etec Crop Solutions) used at 0.05 and 0.075%, Hasten™ (an esterified seed oil, 704 g L^{-1} ethyl and methyl esters of fatty acids from Food Grade canola oil, BASF NZ Ltd) used at 0.5 and 1.0 %, and Li-1000™ (a non-ionic, lecithin+fatty acid esters+alcohol ethoxylate, Batch # 230285, Etec Crop Solutions) used at 0.15 and 0.25%.

Treatments of seven fungicides ± adjuvants were prepared as per label instructions at the rate recommended by the Ministry for Primary Industries (MPI) (Table 1). All treatments mixed easily and were stable at the rates used, and droplets dispensed readily from a micro-syringe. Treatments were applied as 0.5 µl droplets (12 replicates on both abaxial and adaxial leaves from each of the three separate plants) from a micro syringe fitted with a repeating dispenser. All treatments containing Actiwett and Hasten included Blankophore P fluor dye (0.5 % w/v) for droplet visualisation under UV light. All treatments containing Li-1000 included Uvitex NFW fluorescent dye (0.5 %). The fluorescent dyes had no effect on spray solution physical properties. All tests were conducted at 20 ºC and ~55 % relative humidity. Once droplets had dried they were examined under UV light and photographed. Spread areas of droplets were quantified using V++ image analysis software and treatments were compared using analysis of variance (Statistix 10) and least significant difference (LSD) tests.

Figure 1. Adaxial (upper) and abaxial (lower) leaf surfaces of *M. excelsa* plants 1, 2 & 3, used for spread area determination

Table. 1. Fungicides (product and active ingredient) tested in the adjuvant selection trial

Product	Active ingredients (A.I.)	Distributor	MPI use rate ml/500 ml	% w/v
Vandia	triademinol 250 g L^{-1}, EC	Adria NZ	0.25	0.05
Saprol	triforine, 190 g L^{-1}, EC	Orion NZ	0.50	0.10
Timorex Gold	natural extract of Melaleuca alternifolia, 225 g L^{-1}, EC	Syngenta NZ	2.0	0.40
Radial	azoxystrobin+epoxiconazole, 75+75 g L^{-1}, EC	Adama NZ	1.0	0.20
Dedicate	tebuconazole+trifloxystrobin, 200+100 g L^{-1}, SC	Bayer Aust	0.375	0.075
Amistar Xtra	azoxystrobin+cyproconazole, 200+80 g L^{-1}, SC	Syngenta Aust	0.50	0.10
FirstUp	Bio-fungicide XI 115A	BioPharm NZ	2.5	0.50

3.2 Fungicide efficacy trials

The aim of the fungicide efficacy trial was to test three fungicides for their potential to prevent infection of *Metrosideros excelsa* by *A. psidii* .

3.2.1 Plant materials for fungicide efficacy trial

Metrosideros excelsa was used throughout this trial. The *M. excelsa* plants were sourced in 1.5 L pots as small shrubs that were approximately 0.80 m in height. Plants were grown in New Zealand Native Commercial Mix supplemented with; Dolomite 2 kg M^{-3}, Gypsum coarse 2 kg M^{-3}, Lime-Ag grade 2 kg M^{-3}, Permawet 0.75 kg M^{-3}, Triabon 1 kg M^{-3}, Osmocote Exact 12/14 standard start 6 kg M^{-3} and Microplus (TE + Mg + Fe). The plants were kept at the Scion nursery under the shade facility from December 2018 to January 2019 until fungicide was applied.

3.2.2 Establishing spray technique

A spray application technique that used turbulence produced from stationary fans was used to apply fungicide in this trial. This method was established to purposely ensure maximum spray coverage on both the adaxial and abaxial leaf surfaces and also over the whole plant (Fig. 2). A stationary controlled droplet applicator (micromiser5, Micronair, UK) or spinning disc atomiser, here-on referred to as the micromiser5, that produced droplets with a VMD of 130 µm was used for the application of all fungicides. Prior to establishing the application method, several spray trials were conducted to achieve the optimum spraying protocol (these are described in Appendix 1). For all trials, the spraying environment was controlled in that room temperature remained between 18-25 ^{0}C with a relative humidity of 85% and room air movement (outside of the zone of the fans) limited to near-zero. The CDA was powered for spraying via a step-up voltage convertor set at 24 V supported with a motor pump (Rohs Motor pump -12V, 6A, 70W). For the final spray trial, pressure was set at 0.90 MPa, and a flowrate of 300 mL min^{-1} was used to produce small droplets for 40 s to treat *M. excelsa* plants. The micromiser5 was set to operate at 7300 rpm throughout trials.

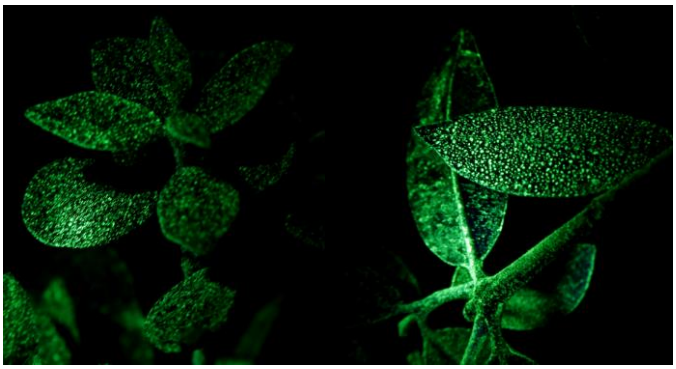

Figure 2. Pictures of sprayed *M. excelsa* plants with Topline Paint (Topline Paint Pty LTD, Australia) showing the coverage achieved on the adaxial (left) and abaxial (right) leaf surfaces with spray methods used.

3.2.3 Fungicide application

Using the method of application described above, and in Appendix 1, three fungicides (Table 3) were applied with adjuvant (Actiwett at 0.75%) on selected *M. excelsa* plants at an equivalent application rate of 1750 L ha^{-1}. These fungicides were selected either because their mode of action is known to be effective (strobilurins & triazoles) or because they are natural product extracts and it is important that we test products outside of conventional fungicides. This application rate was achieved by operating a micromiser5 for 40 seconds at 7300 rpm to produce a flow rate of 300 ml min^{-1}. Droplets with a VMD of 117 µm were produced ensuring excellent coverage of the plant surfaces. During the application, each plant was positioned directly under the nozzle of the micromiser5 which was placed 1.0 m from the ground and 1.0 m distance from the stationary fans producing turbulence. Each fungicide and adjuvant mix were applied on 32 plants with the exception of the control plants that were untreated. Overall 128 plants were used for the trial.

Following spraying, the plants were divided into four different groups with each group inoculated with *A. psidii* at a different time (i.e. inoculation time post-fungicide treatments) either 0, 7, 14 or 21 days after spraying (Table 4). Plants were kept in the shade house until they were taken to the containment facility where they were inoculated with *A. psidii*. The plants were watered via drip irrigation pipes placed directly into the pot throughout the study. Post-inoculation assessment or scoring on disease infection was conducted at 21, 28 and 35 days after inoculation (Table 4) for all inoculated groups (T-0, T-7, T-14 and T-21) of plants.

Table. 3. Fungicide rates used for trial

Active ingredients	Products	Rates (ml L^{-1})	Adjuvant (ml L^{-1})	L ha^{-1}
Tea tree oil	Timorex Gold	2.0	0.75	1.5
Triadimenol+(1-Methyl 2-pyrrollidinon)	Vandia	1.12	0.75	0.84
Azoxystrobin+Epoxiconazole	Radial	0.84	0.75	1.49

Table. 4. Post spraying inoculation times and days for assessment of treated plants

Inoculation time post-fungicide treatments	Assessment days post inoculation		
Time zero (T-0 or Day 0) or plants inoculated a day post-fungicide treatment	21	28	35
Time 7 (T-7 or Day 7) or plants inoculated a week post-fungicide treatment	21	28	35
Time 14 (T-14 or Day 14) or plants inoculated two weeks post-fungicide treatment	21	28	35
Time 21 (T-21 or Day 21) or plants inoculated three weeks days post-fungicide treatment	21	28	35

3.2.4 Spore collection and inoculation procedures

Austropuccinia psidii urediniospores (spores) were collected from already infected *Lophomyrtus bullata* (ramarama) plants into capsules (00 gelatin caps) with the use of a portable vacuum pump (Mini Cyclone Spore Collector GRA-201) powered by a 12V battery. The spores were stored at -80 °C. Prior to inoculation, stored spores were removed from -80 °C and allowed to warm to room temperature. The viability of the spores was assessed following the Tesmann and Dianese (2002) protocol. The concentration of the spores for inoculation was assessed by suspending spores in 0.01 % Tween 20 and counted with hemocytometer. The concentration of the spores was adjusted to $4X10^6$ spores ml^{-1} for inoculation of all *M. excelsa* plants. The inoculation was done on both the adaxial and abaxial leaves for each plant using a manually operated hand sprayer (30 mL spray bottle). The inoculated plants were monitored and kept under controlled conditions of RH (80 - 99 %) at 18-23 ^oC in darkness for 26 hrs. At 26 hrs, the inoculated plants were moved into a controlled environment which had light.

Temperature and relative humidity were recorded and monitored (Appendix 3) during the incubation period in darkness and post-inoculation in the controlled, micro-organism containment facility. The

data collected indicate there was no significant difference between the temperature for all incubation periods for T0, T7, T14 and T21, whereas there were significant differences ($p < 0.05$) between the relative humidity (RH) readings for incubation period in darkness post-inoculation. It is our opinion that these conditions did not affect the outcomes of the trial.

3.2.5 Assessment of disease/infections

The fungicide treated and control *M. excelsa* plants were assessed prior to spraying and at 21, 28 and 35 days post-inoculation. Prior to treating the plants with fungicides, the total number of young shoots on each plant was recorded. Following inoculation, the total number of infected shoots was recorded. Further, for each plant detailed scoring was done for the three most infected shoots. For each of these three shoots we scored the total number of young leaf pairs and the total number of infected leaves. The most infected leaf on each leaf-pair was then labelled and subsequently scored. The scoring of the most infected leaf for each leaf-pair was done for adaxial and abaxial leaf surfaces throughout the study. Further, infected nodal parts of the three most infected shoots were scored. The disease rating scales used for scoring leaves and nodal parts were: 1 = no symptoms evident or presence of yellow flecking; 2 = presence of a hypersensitive reaction (HR) with fleck or necrosis; 3 = small pustules, <0.8 mm diameter, with one or two uredinia; 4 = medium-sized pustules, 0.8–1.6 mm diameter with about 12 uredinia; 5 = large pustules, >1.6 mm diameter, with 20 or more uredinia on leaves, petioles and/or shoots (Junghans et al. 2003) (See Appendix 2 for figures illustrating this scale). In this current study we modified Junghans et al. (2003) scale by introducing a new disease rating scale: 6 = dieback (i.e. dead leaf resulting from infection).

3.2.6 Statistical analysis for fungicide efficacy trials

The trial was designed as a 4 x 4 factorial (4 x 'Inoculation' events X 4' Fungicides' (including control)) arranged in a split plot design with eight replications for each treatment. Inoculation timing was set as the whole plot factor with fungicide (including control plants) set as the sub-plot factor. The design and size of the trial were very constrained by the size of the facility used for conducting the trial under containment prescriptions.

All statistical computations were performed using the statistical analysis and graphics software R version 3.5.2 (R Development Core Team, 2018) with all other details of analyses presented in Appendix 2.

4 Results

4.1 Adjuvants selection trials

Mean values of droplet spreading for replicates over all treatments on plants 1 and 3 (Fig. 3) were 5.11 mm^2 and 4.85 mm^2, respectively, with no significant differences ($P<0.05$) between them. Plant 2 was visibly different (Fig. 3) and treatments spread differently on both surfaces of this plant relative to the other two plants. The overall mean value for replicates on plant 2 was 6.37 mm^2, with no significant differences ($P<0.05$) between them but spreading on plant 2 was significantly different ($P<0.0001$) to plants 1 and 3. This outcome highlights the inherent variability in leaf surface characteristics within a species and the impacts this can have on fungicide spreading, and ultimately performance.

There was a range of spreading exhibited by the seven fungicide formulations; some obviously included more surface-active components (adjuvants) in their formulation than others (Table 5). Saprol, FirstUp and Timorex Gold spread well on adaxial leaf surfaces, and Saprol and FirstUp spread very well on abaxial surfaces (Table 5). On abaxial leaf surfaces, FirstUp and Saprol sprays benefited least from adjuvant addition (Table 5), whereas Timorex Gold benefited somewhat and Vandia, Radial, Dedicate and Amistar Xtra benefited consistently from adjuvant addition (Table 5). Actiwett addition consistently improved spreading of all sprays on at least one leaf surface and often on both (Fig. 3 and Fig. 4). Generally, increasing concentration of Actiwett increased spreading. Overall, there were no significant differences ($P<0.05$) between Hasten and Li-1000 and their concentrations had no effect on spreading (Table 5). Generally, Hasten and Li-1000 improved spreading more on the difficult-to-wet abaxial leaf surface (*cf*. Fig. 3 and 4), but never as much as Actiwett.

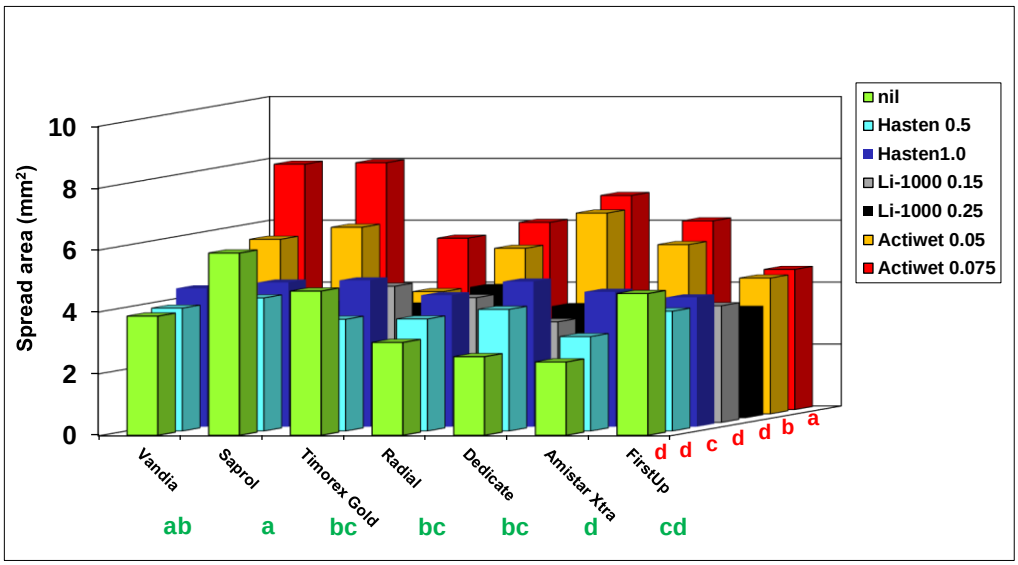

Figure 3. Spreading of fungicide ± adjuvant treatments on *M. excelsa* adaxial leaf surface
Fungicides or adjuvants sharing common colour letters are not significantly different (LSD, P=0.05). LSD (P=0.05) value for comparison between all tmts = 1.03

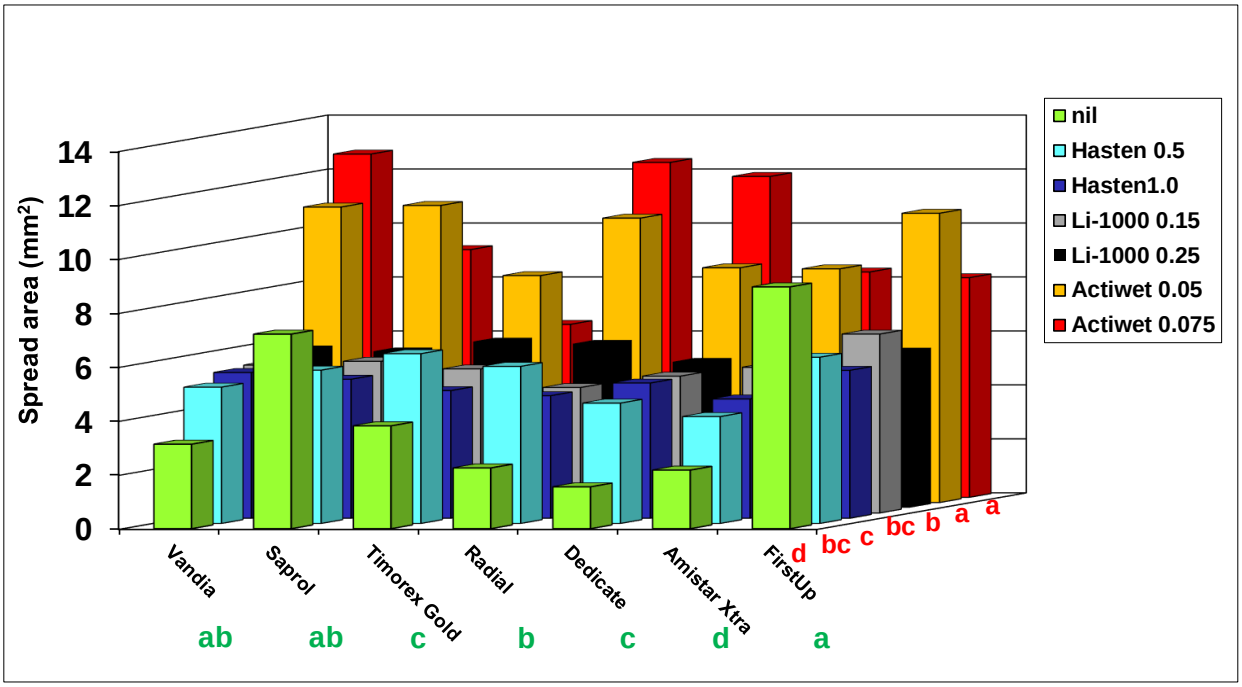

Figure 4. Spreading of fungicide ± adjuvant treatments on *M. excelsa* abaxial leaf surface
Fungicides or adjuvants sharing common colour letters are not significantly different (LSD, P=0.05). LSD (P=0.05) value for comparison between all tmts = 1.37

Table. 5. Spread areas of fungicide ± adjuvant treatments on the upper and lower leaf surfaces of *M. excelsa.* Highlighted cells indicate products used in the fungicide efficacy trials.

Fungicide	Concentration (%)	Adjuvant	Concentration (%)	Spreading on leaf (mm²) adaxial	abaxial
Vandia	0.05	Nil	-	3.85	3.13
		Actiwett	0.05	5.64	10.95
		Actiwett	0.075	7.93	12.72
		Hasten	0.5	3.97	5.05
		Hasten	1.0	4.47	5.39
		Li-1000	0.15	3.54	5.48
		Li-1000	0.25	3.59	5.87
Saprol	0.10	Nil	-	5.89	7.21
		Actiwett	0.05	6.03	11.01
		Actiwett	0.075	7.98	9.18
		Hasten	0.5	4.30	5.68
		Hasten	1.0	4.66	5.15
		Li-1000	0.15	3.60	5.62
		Li-1000	0.25	3.68	5.80
Timorex Gold	0.40	Nil	-	4.66	3.81
		Actiwett	0.05	3.95	8.41
		Actiwett	0.075	5.54	6.41
		Hasten	0.5	3.61	6.29
		Hasten	1.0	4.73	4.73
		Li-1000	0.15	4.40	5.34
		Li-1000	0.25	3.64	6.16
Radial	0.20	Nil	-	3.00	2.25
		Actiwett	0.05	5.35	10.54

Fungicide	Concentration (%)	Adjuvant	Concentration (%)	Spreading on leaf (mm^2) adaxial	abaxial
		Actiwett	0.075	6.05	12.41
		Hasten	0.5	3.62	5.82
		Hasten	1.0	4.25	4.54
		Li-1000	0.15	4.03	4.65
		Li-1000	0.25	4.24	6.08
Dedicate	0.075	Nil	-	2.54	1.55
		Actiwett	0.05	6.49	8.70
		Actiwett	0.075	6.92	11.89
		Hasten	0.5	3.93	4.46
		Hasten	1.0	4.69	5.01
		Li-1000	0.15	3.25	5.07
		Li-1000	0.25	3.62	5.41
Amistar Xtra	0.10	Nil	-	2.37	2.17
		Actiwett	0.05	5.47	8.66
		Actiwett	0.075	6.10	8.35
		Hasten	0.5	3.05	3.96
		Hasten	1.0	4.36	4.42
		Li-1000	0.15	3.01	5.40
		Li-1000	0.25	3.08	4.25
FirstUp	0.50	Nil	-	4.58	8.96
		Actiwett	0.05	4.39	10.72
		Actiwett	0.075	4.53	8.14
		Hasten	0.5	3.87	6.16
		Hasten	1.0	4.18	5.48
		Li-1000	0.15	3.77	6.63
		Li-1000	0.25	3.53	5.79
LSD value for comparison (P=0.05)				1.03	1.37

Values are significantly higher than nil-adjuvant fungicide control

4.2 Fungicide efficacy trials

Leaf disease scores

Infection with *A. psidii* and subsequent sporulation were observed on whole plants, shoots and adaxial (Fig. 5a) and abaxial (Fig. 5b) leaf surfaces, as well as shoot inter-nodes (Fig. 5c) of *M. excelsa* plants. At three weeks post-inoculation infected leaves in both treated and control plants showed dieback (Fig. 5d). The three-way interaction between inoculation time, fungicide treatment and leaf side was not statistically significant, irrespective of assessment date (Table 6). At the first assessment date 21 days after inoculation, the inoculation time × fungicide treatment interaction was statistically significant and a *post-hoc* analysis revealed that the interaction was driven by more pronounced disease symptoms in the control and the Vandia-treated plants compared to the plants treated with Timorex and Radial fungicides when infection took place seven days (i.e. T7) after fungicide application (Table 7 and Fig. 6, see T7 in the top panel). This two-way interaction was near significant at the 28 days (P=0.055) and 35 days (P=0.052) assesments, possibly reflecting the lower infection levels for the inoculation carried out shortly after application (T0). None of the remaining two-way interactions were statistically significant at any of the following assessments. At all assessment dates, disease symptoms were significantly worse on the abaxial compared to the adaxial leaf surface (Fig. 6, Table 6), despite good coverage of fungicides on both leaf surfaces

Figure 5. *Austropuccinia psidii* urediniospores (a) infecting adaxial leaf surfaces (b) infecting abaxial leaf surfaces (c) infecting internode and causing (d) dieback on leaves

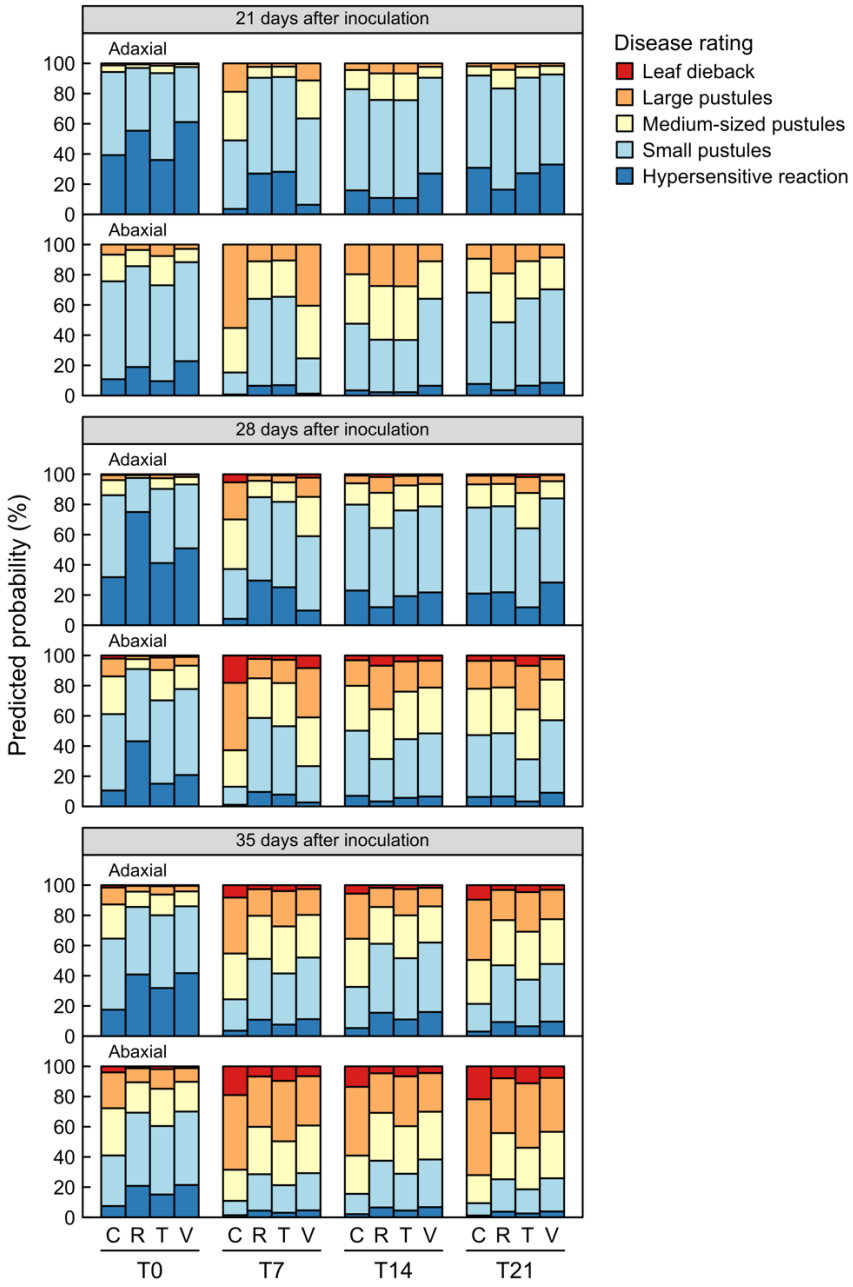

Figure 6. Predicted probabilities from a cumulative link mixed effects model for the leaf disease rating on the adaxial (upper) and abaxial (lower) leaf surface at 21, 28 and 35 days after inoculation (top, middle and bottom panels, respectively). T0 = inoculation of *Metrosideros excelsa* plants with *Austropuccinia psidii* at the same day of the fungicide application, T7, T14 and T21 = inoculation at 7, 14 and 21 days after fungicide application. Fungicide treatments: C = Control, R = Radial + Adjuvant , T = Timorex Gold + Adjuvant, V = Vandia + Adjuvant.

Table 6. Likelihood ratio test results from a backwards selection procedure applied to a cumulative link mixed effects model (CLMM) testing the effects of inoculation time, fungicide treatment, leaf side and their interactions on the leaf disease rating at three assessment dates post-inoculation.

Dropped term	*L*	*df*	*P*	
21 days after inoculation				
First stage				
Inoculation time × fungicide × leaf side	6.35	9	0.705	
Second stage				
Inoculation time × fungicide	19.75	9	0.020	*
Inoculation time × leaf side	3.38	3	0.337	
Fungicide × leaf side	3.71	3	0.294	
Third stage				
Leaf side	46.69	1	<0.001	***
28 days after inoculation				
First stage				
Inoculation time × fungicide × leaf side	4.73	9	0.857	
Second stage				
Inoculation time × fungicide	16.61	9	0.055	
Inoculation time × leaf side	1.16	3	0.762	
Fungicide × leaf side	1.43	3	0.697	
Third stage				
Inoculation time	4.37	3	0.224	
Fungicide	5.96	3	0.114	
Leaf side	39.07	1	<0.001	***
35 days after inoculation				
First stage				
Inoculation time × fungicide × leaf side	4.38	9	0.885	
Second stage				
Inoculation time × fungicide	16.80	9	0.052	
Inoculation time × leaf side	0.70	3	0.873	
Fungicide × leaf side	2.93	3	0.402	
Third stage				
Inoculation time	5.82	3	0.121	
Fungicide	4.81	3	0.187	
Leaf side	21.45	1	<0.001	***

L = likelihood ratio statistic, *df* = degrees of freedom of the *L* statistic, *P* = *P*-value. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 7. Results from a *post-hoc* analysis contrasting the fungicide treatments within inoculation times associated with the CLMM for the assessment 21 days post-inoculation (slicing the significant inoculation time × fungicide interaction).

Contrast	Estimate	SE	*z*	$P_{adj.}$	
Inoculation time T0					
C – R	0.70	1.06	0.66	0.761	
C – T	-0.07	0.63	-0.12	0.906	
C – V	0.93	0.74	1.26	0.621	
R – T	-0.77	1.06	-0.73	0.761	
R – V	0.23	1.14	0.20	0.906	
T – V	1.01	0.78	1.29	0.621	
Inoculation time T7					
C – R	2.36	0.61	3.85	<0.001	***
C – T	2.44	0.59	4.14	<0.001	***
C – V	0.62	0.60	1.04	0.358	
R – T	0.08	0.58	0.14	0.892	
R – V	-1.73	0.67	-2.58	0.015	*
T – V	-1.82	0.62	-2.93	0.007	**
Inoculation time T14					
C – R	-0.38	0.72	-0.53	0.898	
C – T	-0.32	1.39	-0.23	0.966	
C – V	0.73	0.79	0.92	0.824	
R – T	0.06	1.47	0.04	0.966	
R – V	1.11	0.93	1.20	0.824	
T – V	1.05	1.28	0.82	0.824	
Inoculation time T21					
C – R	-0.85	0.76	-1.12	0.692	
C – T	-0.13	0.60	-0.23	0.822	
C – V	0.14	0.53	0.27	0.822	
R – T	0.71	0.76	0.94	0.692	
R – V	0.99	0.74	1.33	0.692	
T – V	0.27	0.62	0.44	0.822	

Estimate = estimated difference, SE = standard error of the difference, *z* = *z*-value (infinite degrees of freedom), P_{adj} = multiplicity adjusted *P*-value (Banjamini & Hochberg method (1995).

Percentage leaf infection

None of the interaction terms nor the main effects of inoculation time or assessment date produced statistically significant effects (Table 8). A significant fungicide main effect was driven by consistently lower probabilities of infected leaves associated with the Radial fungicide (Figure 7, Table 8). The probabilities of infected leaves related to the remaining two fungicides neither differed from the control nor from each other (Figure 7, Table 9). In summary, the timing of inoculation did not have an effect on the percentage of leaves infected and, out of all the fungicides tested, only Radial reduced the leaves infection.

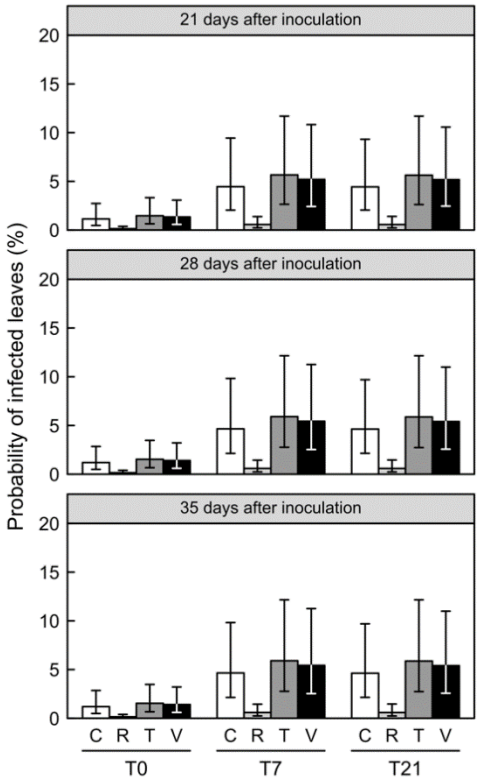

Figure 7. Predicted probabilities from a generalised linear mixed effects model applied to the proportion of infected leaves at 21, 28 and 35 days after inoculation (top, middle and bottom panels, respectively). T0 = inoculation at the same day of the fungicide application, T7 and T21 = inoculation at 7 and 21 days after fungicide application. Fungicide treatments: C = Control, R = Radial + Adjuvant , T = Timorex Gold + Adjuvant, V = Vandia + Adjuvant. Error bars indicate standard errors..

Table. 8. Likelihood ratio test results from a backwards selection procedure applied to a generalised linear mixed effects model (GLMM) testing the effects of inoculation time, fungicide treatment, assessment date and their interactions on the proportion of infected leaves

Dropped term	*L*	*df*	*P*
First stage			
Inoculation time × fungicide × assessment date	0.20	12	1
Second stage			
Inoculation time × fungicide	5.70	6	0.458
Inoculation time × assessment date	0.18	4	0.996
Fungicide × assessment date	0.10	6	1
Third stage			
Inoculation time	3.87	2	0.145
Fungicide	7.93	3	0.048 *
Assessment date	0.18	2	0.915

L = likelihood ratio statistic, *df* = degrees of freedom of the *L* statistic, *P* = *P*-value. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table. 9. Results from a *post-hoc* analysis contrasting the fungicide treatments associated with the GLMM for the leaf infection data.

Contrast	Estimate	SE	*t*	$P_{adj.}$	
C – R	2.10	0.89	2.35	0.038	*
C – T	-0.25	0.83	-0.30	0.914	
C – V	-0.16	0.83	-0.20	0.914	
R – T	-2.35	0.89	-2.65	0.033	*
R – V	-2.26	0.89	-2.55	0.033	*
T – V	0.09	0.83	0.11	0.914	

Estimate = estimated difference, SE = standard error of the difference, *t* = *t*-value (819 degrees of freedom), P_{adj} = multiplicity adjusted *P*-value (Benjamini & Hochberg method (1995)). Results are averaged over the levels of inoculation time and assessment dates. Results are given on the log odds ratio scale.

Percentage shoot infection

(Note the Day-14 Inoculation results were omitted from this analysis-see Appendix 2).

Neither the inoculation time × fungicide × assessment date interaction nor any of the three two-way interactions was statistically significant (Table 10). The predicted proportion of infected shoots was generally somewhat higher in the control group compared to the fungicide-treated plants, which resulted in a significant fungicide treatment main effect (Table 10). A *post-hoc* analysis showed that these differences were only statistically significant between the control plants and those treated with the Radial fungicide (Figure 8). The assessment date did not have a significant effect, but the inoculation times differed significantly from each other (Figure 8, Table 10). A *post-hoc* comparison revealed that inoculation time T0 had significantly lower probabilities of infected shoots compared to inoculation time T7 and T21, which produced similar shoot infection probabilities (Figure 8, Table 11).

Table 10 Likelihood ratio test results from a backwards selection procedure applied to a generalised linear mixed effects model (GLMM) testing the effects of inoculation time, fungicide treatment, assessment date and their interactions on the proportion of infected shoots. *L* = likelihood ratio statistic, *df* = degrees of freedom of the *L* statistic, *P* = *P*-value. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Dropped term	*L*	*df*	*P*
First stage			
Inoculation time × fungicide × assessment date	2.61	12	0.998
Second stage			
Inoculation time × fungicide	7.36	6	0.288
Inoculation time × assessment date	1.95	4	0.745
Fungicide × assessment date	1.49	6	0.960
Third stage			
Inoculation time	9.36	2	0.009 **
Fungicide	9.38	3	0.025 *
Assessment date	0.72	2	0.699

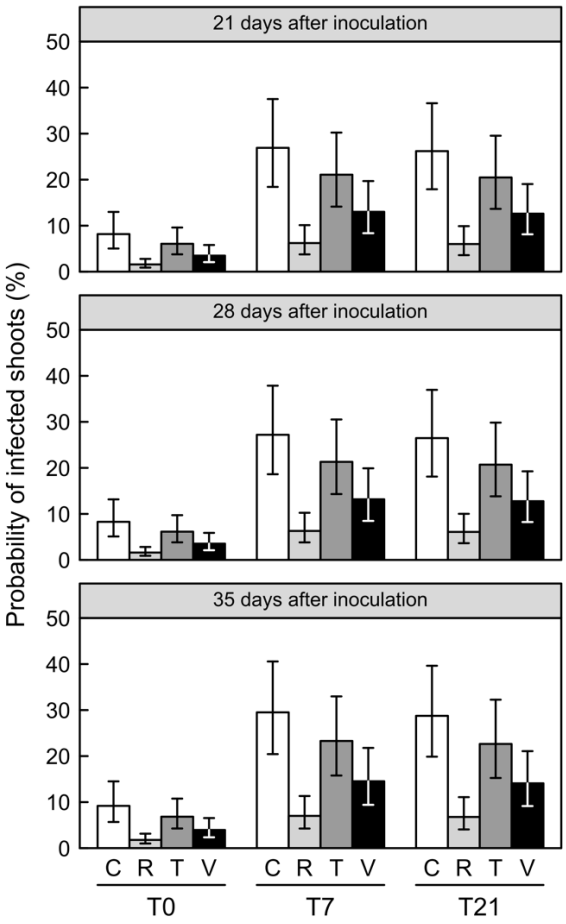

Figure 8. Predicted probabilities from a generalised linear mixed effects model applied to the proportion of infected shoots at 21, 28 and 35 days after inoculation (top, middle and bottom panels, respectively). T0 = inoculation at the same day of the fungicide application, T7 and T21 = inoculation at 7 and 21 days after fungicide application. Fungicide treatments: C = Control + Adjuvant, R = Radial + Adjuvant , T = Timorex Gold + Adjuvant, V = Vandia + Adjuvant. Error bars indicate standard errors.

Table 11 Results from a *post-hoc* analysis contrasting the fungicide treatments associated with the GLMM for the shoot infection data. Estimate = estimated difference, SE = standard error of the difference, t = t-value (277 degrees of freedom), P_{adj} = multiplicity adjusted P-value (Benjamini & Hochberg method (1995)). Results are averaged over the levels of fungicide treatment and assessment dates. Results are given on the log odds ratio scale.

Contrast	Estimate	SE	t	$P_{adj.}$	
T0 – T7	-1.42	0.51	-2.81	0.010	*
T0 – T21	-1.38	0.51	-2.73	0.010	*
T7 – T21	0.04	0.48	0.08	0.939	

4.3 Outcomes

The trial to test different adjuvants to improve spread of fungicides on *M. excelsa* leaves was very successful. The spread/coverage of the fungicide sprays, with and without the addition of three different classes of adjuvants was quantified on both (i.e. adaxial and abaxial) leaf surfaces of *M. excelsa*. The addition of adjuvants to fungicide for application is likely to benefit the adhesion, retention and coverage of fungicide sprays applied to *M. excelsa* trees and will thus help optimise their

efficacy. The adjuvant Actiwett, an alcohol ethoxylate, proved to be the best among the three adjuvants tested. Although Actiwett was tested or screened for the spread of seven different fungicides, only three of the fungicides were used for the main trial testing efficacy of products for control of myrtle rust. The limitation to three fungicides was due to constraints around space in our PC1 containment facility.

The development of a spray application technique based on the use of turbulence to maximize the spread and deposition of fungicides on both the adaxial and abaxial leaf surfaces yielded very promising results in this study and was a good method to ensure that poor coverage was not an issue in our controlled efficacy trial (details in Appendix 1). For the first time, an innovative and critically thought-through technique has been established using a micromiser5 for fungicide spray trials.

After successfully developing the myrtle rust inoculation protocol for the first time in New Zealand, our research focused on a preventative fungicide trial using only *M. excelsa* as a host plant. The treated plants responded differently to myrtle rust infection depending on the inoculation time and fungicide type applied. Generally, the infected leaves and shoots were higher in the controls than fungicide treated *M. excelsa* plants. The only fungicide that significantly controlled *A. psidii* infection in *M. excelsa* shoots compared to the control was Radial. The efficacy of the fungicide, however, declined over time, with higher infection levels observed for those plants that were infected 7, 14 and 21 days after infection (see Figures 6, 7 and 8). This outcome is related to the relatively short activity (or persistence) of the fungicides tested. It would be expected that this outcome would be possibly shorter in a field situation.

Importantly, despite good coverage of fungicide achieved on both leaf surfaces for all treatments, the infection levels on the abaxial leaf surface was consistentialy significantly higher than that on the adaxial surface, as would be expected for this pathogen.

5 Conclusions

After successfully completing the first phase of the pilot chemical control trials for myrtle rust in New Zealand, the following conclusions have been made:

1. The adjuvant, Actiwett, an alcohol ethoxylate was shown to enhance the spread of seven fungicides on *M. excelsa* plants.
2. A successful inoculation protocol for infecting New Zealand Myrtaceae has been established in New Zealand. This will be useful for future trials that will require artificial inoculation protocols for studies on the ecology and management of myrtle rust.
3. Comparatively, among the three fungicides used in the preventative trial study, Radial significantly controlled *A. psidii* on *M. excelsa* plants. The efficacy of the fungicide appeared to decline over the period of the trial with higher infection levels observed as time from spraying increased.
4. Despite good fungicide coverage on both leaf surfaces, infection on the abaxial surface was consistently higher than that on the adaxial surface.
5. Urediniospores that infected the host plant were not the only life-cycle stage of the *A. psidii* pathogen identified or observed during the trials. For the first time, teliospores were identified on some of the infected leaves.

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Appendix 1. Protocol development

Before arriving at a final spraying protocol, a number of trials were conducted in the Scion tracksprayer to determine the set-up that would provide the best coverage on the test plants using the micromiser5. The main studies are described below.

Experiment 1: Effect of exposure time and flow rate in presence of turbulence on spray deposition, coverage and recovery from steel plates

Artificial targets were made using four steel plates (76 x 152 mm) mounted at four heights on a central steel rod, with consecutive plates oriented in different directions to reduce shading of one plate by another. The bottom plate was close to ground level and there was a 20 cm vertical separation between consecutive plates (i.e. top plate was at approximately 80 cm above ground level). The selected segment distance between plates was based on similar segments for sampling leaves from the plant in Experiment 1. One voltage (24 V) was used to operate the spraying system throughout this trial. The experiment was repeated three times per set up for plates sprayed for 20, 40 and 60 s, all at a rate of 150 ml min^{-1} (Table 1a). This rate was selected because at 300 ml min^{-1} the run-off was excessive on the plates. Overall, a total of 9 treatments (three set-up replications for 20, 40 and 60 s spray time) each with 4 plates making a total of 36 plates were used for the trial. The four plates per spray trial were labelled P1 (plate at ground level), P2 (above P1), P3 (above P2) and P4 (the crown or above P3). The plates were collected shorty after spray application. The collected plates were sent to the laboratory where subsequent wash-offs were carried out. As per previous trial the spray mix was made up of water containing 0.01 kg L^{-1} tartrazine, a light stable colorimetric tracer (Hawkins Watts Ltd., New Zealand). Spray deposition was quantified on steel plates by washing the dye from the samples using a 100 ml distilled water and applying standard colorimetric techniques (Richardson et al. 1989) to measure light absorbance of the sample at l max. 427 with a spectrophotometer (T70 UV/VIS, PG Instruments Ltd, UK). Deposition was quantified with reference to the concentration of tracer in a tank sample from the spray mix applied.

Table 1a. Summary of treatments applied to steel plates.

Treatment code	M5 voltage	Flow rate (ml min^{-1})	Spray duration (s)	Spray applied (ml)	Nominal application rate (L ha^{-1})
T1	24	150	20	50	442.0
T2	24	150	40	100	884.1
T3	24	150	60	150	1326.1
T4	24	150	20	50	442.0
T5	24	150	40	100	884.1
T6	24	150	60	150	1326.1
T7	24	150	20	50	442.0
T8	18	150	40	100	884.1
T9	24	150	60	150	1326.1

[a] Nominal application rate was calculated assuming the M5 effective swath is 1.2 m diameter.

Experiment 2: Effect of exposure time and flow rate in presence of turbulence on spray deposition, coverage and recovery from *M. excelsa* foliage

M. excelsa plants were selected for the trial. All the plants were put in a stationary position under the micromiser5 (Micronair, UK) spinning disc atomiser as described in the section Establishing a Spraying Technique. Two-operating stationary electric fans were elevated to a height of 0.3 m from the ground adjacent to each other with the plant positioned at the center of both fans, 1 m away from both fans. The plant was positioned directly below the micromiser5 which was approximately1 m from the ground. A spray mix containing tartrazine, a light stable colorimetric tracer, at 0.01 kg L^{-1} was applied to the plants with the micromiser5 (Hawkins Watts Ltd., New Zealand). Application volume was varied by testing three different flow rates (150, 200 and 300) ml min^{-1} applied at five different

exposure timings (10, 20, 30, 40 and 60) at varying voltages (18 and 24V) supplied to the micromiser5, which had the effect of altering the disc rpm (data not shown) (Table 1b).

The sprayed plants were left to stand for some few minutes to allow spray deposition to settle on leaves of *M. excelsa* plants. The height of each plant was then divided into four parts (segments) about 20 cm apart as: ground, above ground, close-to-crown, and crown level. Three leaves were sampled from each segment, making a total of twelve leaves per plant. The sampled leaves from each of the four parts per plant were put into storage bags and sent to the Pest Management laboratory at Scion where they were analysed for coverage (Fig. A1).

Spray deposition was quantified on leaf surfaces by washing the dye from the samples using 50 ml distilled water and applying standard colorimetric techniques (Richardson et al. 1989) to measure light absorbance of the sample at l max 427 with a spectrophotometer (T70 UV/VIS, PG Instruments Ltd, UK). Deposition was quantified with reference to the concentration of tracer in a tank sample from the spray mix applied. Subsequently, the total one-sided leaf area from each section of each plant was quantified by Imaging J Software.

Table 1b. Summary of treatments applied to *M excelsa* foliage.

Treatment code	M5 voltage	Flow rate (ml min^{-1})	Spray duration (s)	Spray applied (ml)	Nominal application rate (L ha^{-1})
PT1	24	300	10	50	442.0
PT2	24	300	20	100	884.1
PT3	24	300	30	150	1326.1
TP2	24	150	40	100	884.1
TP3	24	150	60	150	1326.1
TP4	24	300	40	200	1768.2
TP5	24	200	40	133	1178.8
TP6	18	150	40	100	884.1
TP7	24	300	40	200	1768.2

[a] Nominal application rate was calculated assuming the M5 effective swath of 1.2 m diameter.

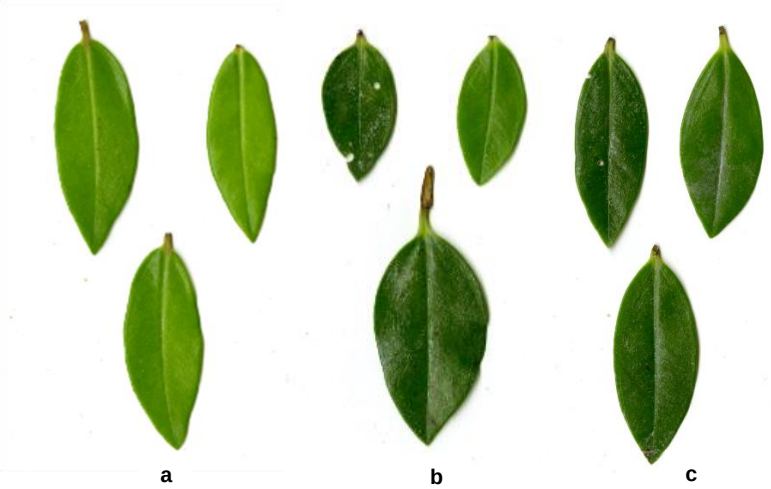


Fig. A1. Pictures showing treatment leaf-features after washing-off tartrazine for (a) Crown leaves (b) Close-to-crown leaves (below crown part) and (c) above-ground leaves.

Data analysis of spray deposition

Spray deposition per unit area was calculated for both steel plates (Fig. 3) and leaf samples (Fig. 4). To account for treatment differences in the amount of spray applied, deposition data were also normalised to calculate deposition per 100 ml of spray applied. Analysis of covariance (SAS GLM

procedure) was used to determine the effect of application rate and sampling height on spray deposition and normalised spray deposition on both steel plates and leaves sampled from treated *M. excelsa* plants.

Results

Spray recovery from steel plates

Averaging across all heights, there was no significant effect of application rate on spray deposition ($p = 0.15$) (Fig. A2a) but a highly significant negative linear relationship between normalised deposition and the volume of spray applied ($p = 0.0007$) (Fig. A2b). This result is unexpected and implies that as the amount of spray applied increased, retention of spray on plates decreased. Assuming an effective circular swath diameter of 1.2 m, actual application rates ranged from 442 to 1326 L ha^{-1} (Appendix 1: Table 1a). These are high rates and increasing run-off from the plates as application rates increased is a plausible explanation, especially with the addition of a significant airstream from the fans and the rigid plate disposition. It is also known that droplets impacting on an already wetted surface tend to bounce, increasing the likelihood of losses, with the applied airstream also potentially increasing the displacement of any bouncing droplets away from the target.

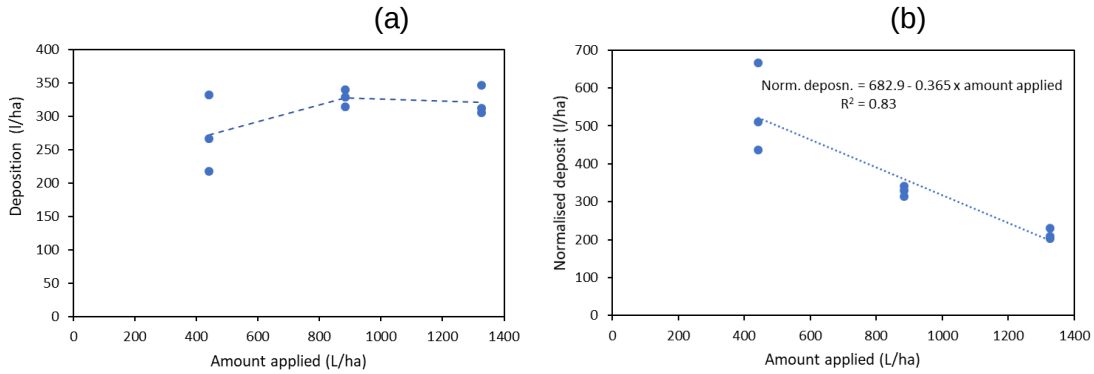


Fig. A2. Relationship between (a) actual and (b) normalised spray deposition on steel plates and the total amount of spray applied

Average deposition across all steel plate data was 307 L ha^{-1} (standard deviation of 261 L ha^{-1}). However, there was a significant effect of sampling height on both actual and normalised spray deposition ($P < 0.0001$) (Fig. A3a and A3b) with highest deposition at the highest and third plate (positions 4 and 2) and lowest deposition on the bottom plate. While it is conceivable the shading of one plate by another could have reduced deposition on plate 2 relative to plate 4 and plate 1 relative to plate 3 because of their locations, in reality this explanation is unlikely given plates at successive heights were deliberately positioned to minimise shading from higher plates. It is possible, and more likely, that the distribution of spray was not homogenous within the swath, particularly with the influence of the fans on droplet trajectories. Further, the distribution of air velocity from the fans would not have been uniform also potentially contributing to the observed results. If that is the case, treatment uniformity could be improved by rotating the targets on their central axis during spraying, so all parts of the target are exposed to possible regions of higher or lower sprayer concentration and air velocity. Using a rotating platform could be tested in future trials.

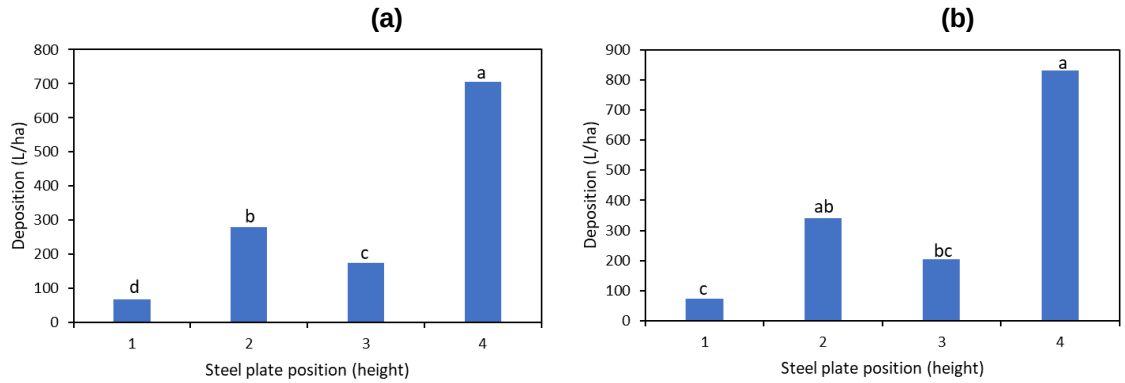


Fig. A3. Effect of sampling height on (a) actual and (b) normalised spray on steel plates averaged across all treatments; Height position 4 represents the top plate and 1 the lower plate.

Spray recovery from *M. excelsa* foliage

Averaging across all heights, there was a significant and positive effect of application rate on spray deposition ($p = 0.0009$) (Figure A4a), but no significant relationship between normalised deposition and the volume of spray applied ($P = 0.546$) (Figure A4b). This result contrasts to the plate data and the factors causing lower spray retention on plates as application volume increased are not influencing deposition on leaves. There are three potential explanations for this effect:

(i) The micro-roughness or wettability characteristics of the two surfaces increase the likelihood of losses from steel plates relative to foliage. This explanation is unlikely given the very small droplet sizes (increases likelihood of adhesion) and no evidence of losses of much larger droplets from plates in many previous studies.

(ii) The application of airstreams from fans has increased the likelihood of losses from plates compared with foliage. This explanation is plausible. Plates are a rigid surface that do not absorb any energy from the airstream whereas the plant surfaces move and absorb energy.

(iii) If spray is lost from any of the plates due to runoff or blow-off, it is likely to be lost from the sampling system due to the way the plates were oriented in space. However, if spray is lost from any leaf surface there is some level of probability that it will be recaptured by a leaf randomly located lower in the crown profile.

Regardless of the explanation, this result indicates the proposed treatment method is effective at capturing and retaining a high proportion of the applied spray.

Average deposition across all foliage data was 292 L ha^{-1} (standard deviation of 196 L ha^{-1}). In contrast to the plate data, there was no significant effect of sampling height on actual or normalised spray deposition (Figure A5) ($p > 0.05$), although there was a trend for less deposition at lower crown levels. This result is not an unexpected given the highly variable nature of crown architecture and the relatively low foliage area densities of the test plants. While the steel plate data suggested that spray distribution may not have been uniform, the random sampling of the foliage elements (i.e. not adhering to the strict positional consistency of the steel plate samples), presumably avoided issues of positional sample bias at the different sampling heights.

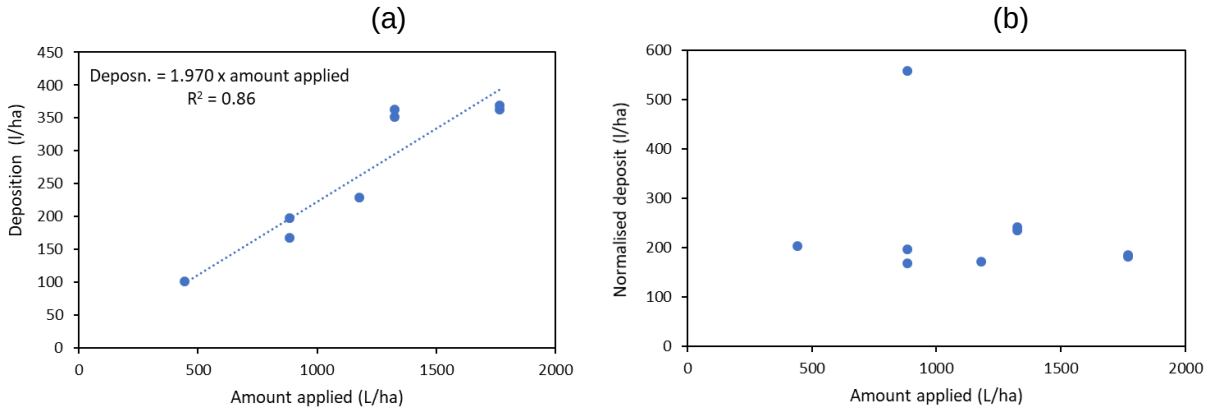


Fig. A4. Relationship between (a) actual and (b) normalised spray deposition on foliage and the total amount of spray applied

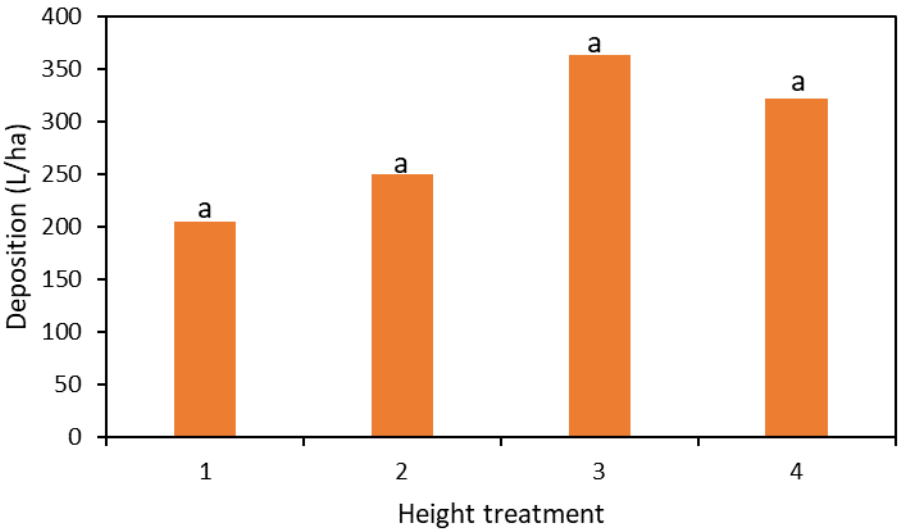


Fig. A5. Effect of sampling height on spray deposition on foliage averaged across all treatments; Height position 4 represents the crown and 1 the lower tier of leaves sampled.

Conclusions and recommendations from the preliminary spray trials

- Despite the lower retention of spray on plates at high application volumes, there was not a significant difference in average deposition on steel plates and foliage (307 versus 292 L ha^{-1} respectively, with high variance).
- The even distribution of spray with height through the plant crowns indicates good crown penetration using this methodology. While attenuation may be more marked when using plants with higher foliage area density, this methodology appears to be effective.
- The steel plate sampling system was ineffective for this experimental design with reduced spray retention as application rates increased. Although the precise reason for poor spray retention is not known it may have been due to interaction with the rigid steel plates and the airflow from the fans.
- There was evidence of large and consistent spatial variance in spray distribution that may have resulted in sampling bias for the steel plate data. Such bias could explain the observed effect of sampling height on spray deposition and the fact that despite demonstrable poor retention at high application rates on plates, average deposition on plates and foliage were not different.
    - It would be useful to experimentally test the hypothesis of consistent spatial variance in spray deposition.
    - Placing plants on a rotating turntable would help reduce the effects of such spatial bias.

Appendix 2. Disease rating scale and analyses used for our study

Disease Rating Scores

The disease rating scales used for scoring leaves and nodal parts were: 1 = no symptoms evident or presence of yellow flecking; 2 = presence of a hypersensitive reaction (HR) with fleck or necrosis; 3 = small pustules, <0.8 mm diameter, with one or two uredinia; 4 = medium-sized pustules, 0.8–1.6 mm diameter with about 12 uredinia; 5 = large pustules, >1.6 mm diameter, with 20 or more uredinia on leaves, petioles and/or shoots

Details of the statistical analyses

Leaf and nodal disease scores

The leaf and nodal disease scores were analysed using cumulative link mixed models (CLMM) with logit link fitted by Laplace approximation (R package *ordinal*, Christensen 2019). Inoculation time (0, 7, 14 and 21 days after fungicide application), fungicide treatment (Control, Radial + Adjuvant, Timorex Gold + Adjuvant, Vandia + Adjuvant), leaf surface (adaxial, abaxial) and their interactions formed the fixed effects term of the model. The random term reflected the multilevel study design with the following nesting structure from the highest to the lowest level for the leaf disease model: Block/inoculation time/fungicide treatment/leaf surface/plant/shoot number. The random term of the nodal disease model had the following structure: block/inoculation time/fungicide treatment/plant. The overwhelming majority of the foliage was symptom-free over time (82.3% of all observations) and therefore the analysis was restricted to diseased leaves. Due to model convergence issues (overparameterisation), separate CLMMs were run for the three assessment dates (21, 28 and 35 days after inoculation). A comparison of models with identical fixed and random terms but different threshold options (flexible, equidistant, two varieties of symmetric thresholds) based on the Akaike Information Criterion (AIC) indicated that flexible thresholds were best suited for the leaf disease score data (ΔAIC > 10 in favour of the CLMM with flexible thresholds). For the nodal disease score data CLMMs showed minor differences between flexible, equidistant and symmetric thresholds (ΔAIC < 3) and we therefore opted for the default flexible thresholds.

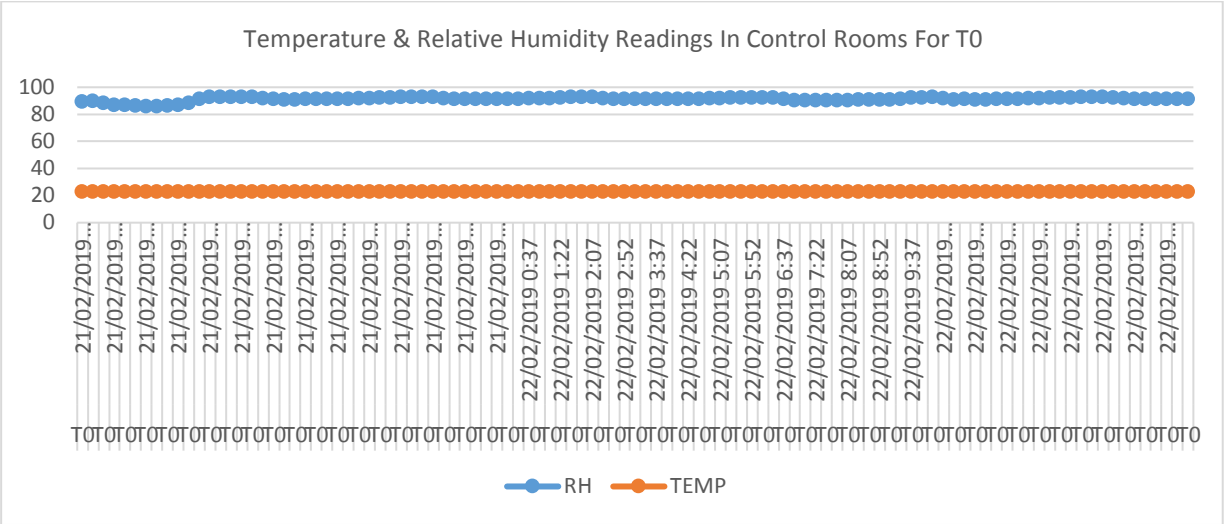
Percentage leaf and shoot infection

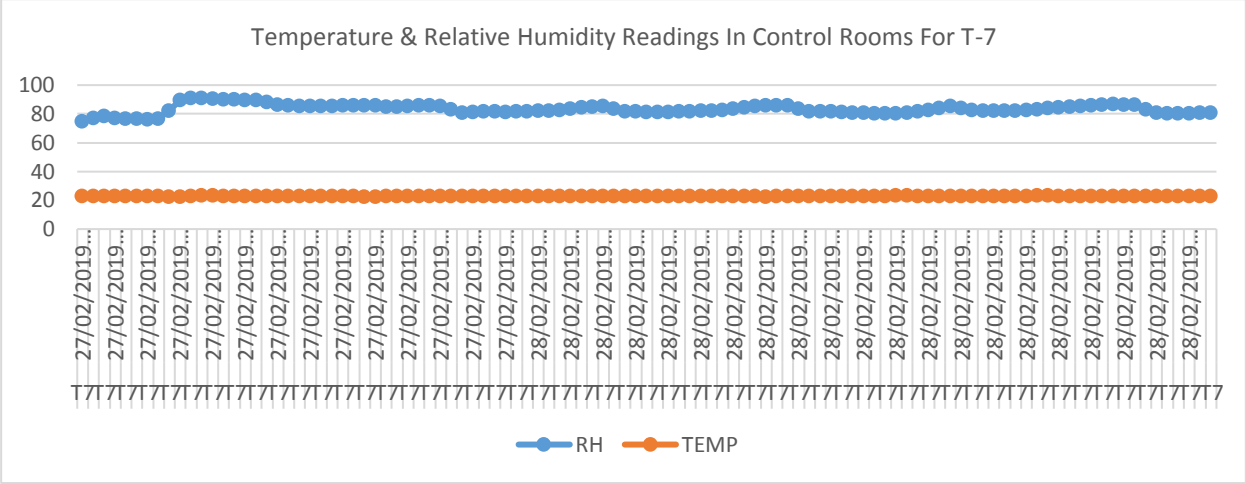
As the data underlying the percentages of infected leaves and shoots were available, we applied a generalised linear mixed effects model (GLMM) with binomial errors and logit link using maximum likelihood estimation (R package *glmmTMB*, Brooks et al. 2017). The binomial response was specified by a two-column matrix holding the number of infected leaves (or shoots) and the number of uninfected leaves (or shoots). The fixed component comprised inoculation time, fungicide treatment, assessment date and their interactions. The random term of the leaf GLMM consisted of the following top-down nesting structure: block/inoculation time/fungicide treatment/plant. Since there was no sub-replication beyond the shoot level, the random term of the GLMM applied to the shoot data only consisted of the nested term: block/inoculation time/fungicide treatment. The observations associated with inoculation time T14 were omitted from the analysis as the low infection observed for this date was overwhelming the analysis.

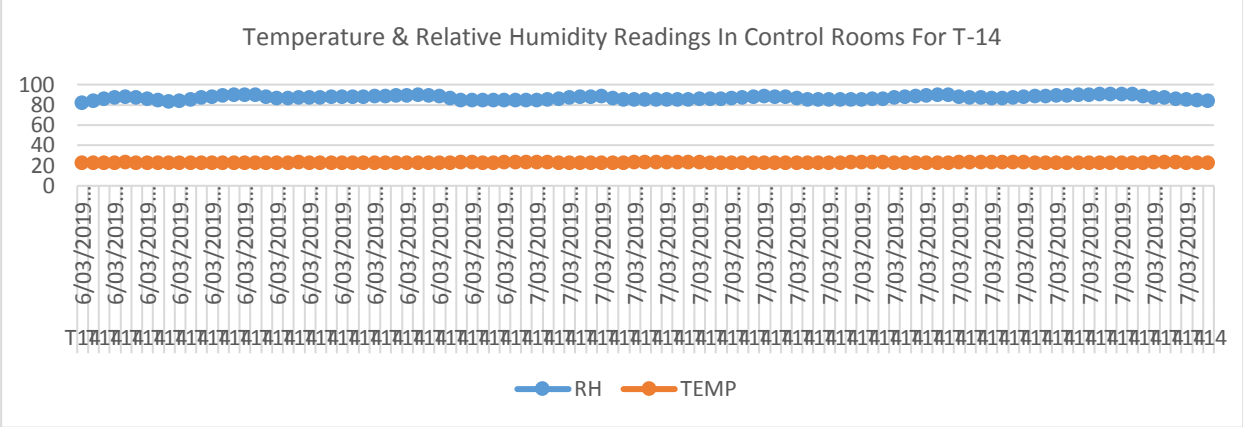
For all models, the significance of the explanatory variables was assessed using a backwards selection approach based on likelihood ratio tests. Statistically significant effects were followed up with a *post-hoc* analysis using the Benjamini & Hochberg (1995) multiplicity adjustment (R package *emmeans*, Lenth 2019).

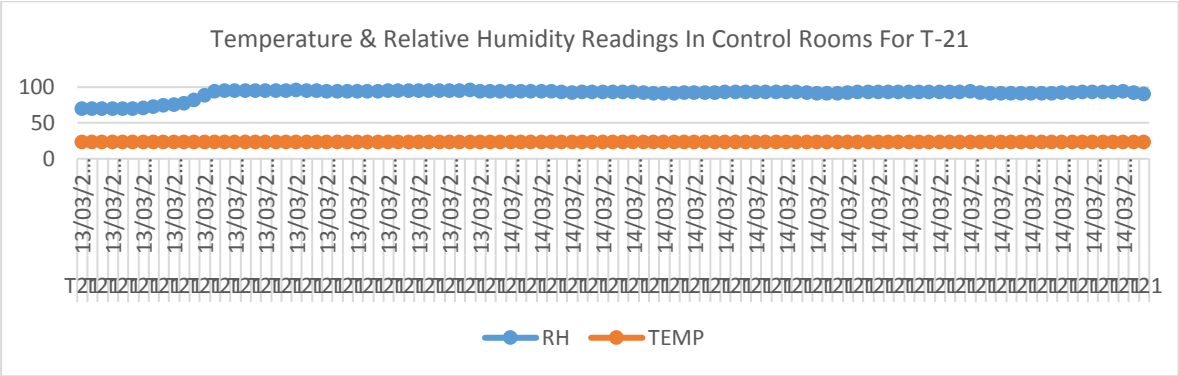
Appendix 3. Temperature and RH conditions

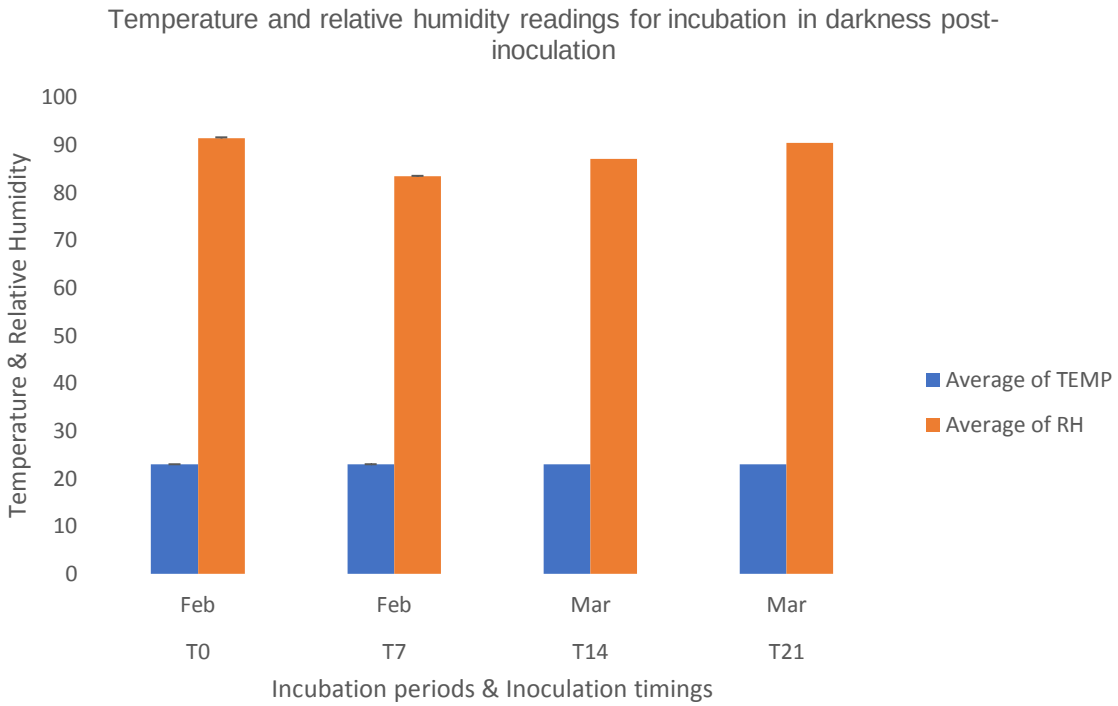
Temperature and relative humidity readings in control rooms during incubation post-inoculation period for T0, T7, T14 & T21 respectively











Temperature and relative humidity readings in Containment Room during incubation period in darkness post-inoculation for all inoculation times. Bars with the same letters are not significantly different (P <0.05) from each other based on Tukeys test.