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Exploring the efficacy of alternative products to control myrtle rust

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Executive summary

Exploring the efficacy of alternative products to control myrtle rust

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Myrtle rust, caused by *Austropuccinia psidii*, poses a serious threat to some native and exotic species in the Myrtaceae (myrtle family) in New Zealand and there is urgent need to develop management options for controlling this disease. This project, funded by the Ministry for Primary Industries (MPI), sought to evaluate alternatives to conventional synthetic fungicide for control of myrtle rust. A series of glasshouse (The New Zealand Institute for Plant and Food Research Limited (PFR), Ruakura & Lincoln) and outdoor experiments (PFR, Pukekohe) were conducted on potted plants on two myrtaceous species, *Lophomyrtus* sp. cultivar 'Red Dragon' (*L. bullata* x *L. obcordata* natural hybrid) and *Metrosideros excelsa* (pōhutukawa).

Fifteen different control agents, including 'soft chemicals', microbial agents and plant defence inducers, were evaluated in glasshouse experiments at Ruakura. The commercial biofungicide, Bacstar ®, was selected as a positive control based on efficacy data obtained in related studies. Bacstar reduced myrtle rust severity on lophomyrtus by 41–77% (across nine experiments) but was less effective (27%) on pōhutukawa. The most effective treatment was a commercially available product, Kiwicare Organic Super Sulphur, which reduced myrtle rust severity by 97% on lophomyrtus and by 59% on pōhutukawa. Of the new 'alternatives', the most promising were four different water-soluble chitosan fractions, which reduced myrtle rust by up to 80% on lophomyrtus and by 38% on pōhutukawa depending on concentration and source material. The essential oils, aniseed oil, lemongrass oil and rosemary oil reduced myrtle rust by 20–30% on average on lophomyrtus and pōhutukawa. Similarly, sodium bicarbonate and potassium bicarbonate, and putative defence inducers, cinnamic acid and salicylic acid, reduced myrtle rust by 28–38% on lophomyrtus but had no significant effect on myrtle rust severity on pōhutukawa.

Of the agents tested in the glasshouse assays at PFR, Ruakura, Bacstar, aniseed oil, rosemary oil, sodium bicarbonate, potassium bicarbonate and chitosan oligosaccharides (PB-2 & PB-4) were selected for further evaluation in outdoor potted plant trials (see below and Section 4).

At PFR, Lincoln, three novel bacterial antagonists isolated from healthy myrtaceous plants were tested on *Lophomyrtus* sp. 'Red Dragon', initially as detached twigs (twiglet assay) and then as young potted plants. All bacterial antagonists were initially tested alone and in different combinations against myrtle rust, using a twiglet assay in vitro. In vitro experiments suggested that bacterial antagonists worked better as curative agents against myrtle rust infection, with the *Bacillus* sp. strain 776 alone, *Pseudomonas* sp. strain 881 alone and the combination of *Pseudomonas* sp. strain 881 and *Serratia* sp. strain 1067 being the most effective. Their efficacy was like that of the commercial biocontrol agent

Bacstar. The three bacterial antagonists and the combination of *Pseudomonas* sp. strain 881 and *Serratia* sp. strain 1067 were then tested in the glasshouse on young potted plants, using the bacterial antagonists as curative agents. The results showed that when *Bacillus* sp. strain 776 and the combination of *Pseudomonas* sp. strain 881 and *Serratia* sp. strain 1067 were applied after infection by myrtle rust, they significantly reduced rust infection relative to the positive control (rust only) by 54% and 49%, respectively, compared to 35% reduction by Bacstar. The positive effect of the bacteria was most evident in the new plant tissue produced during the experiment. The dynamics of the bacterial antagonists on the leaf surface was examined using metabarcoding. Metabarcoding analysis demonstrated that genera encompassing the bacterial antagonists had greater relative abundance on the leaf surface after they were inoculated, suggesting that these strains had survived and colonised the leaf surface in the presence of myrtle rust.

Further research is needed to understand the commercial potential of the promising bacterial strain (*Bacillus* sp.) for myrtle rust control in the field.

Outdoor experiments on potted *Lophomyrtus* sp. 'Red Dragon' plants at PFR, Pukekohe compared the efficacy of readily available commercial fungicides that are registered for use in New Zealand by the Agricultural and Veterinary Medicines Group of Ministry for Primary Industries (ACVM) with the most promising products identified in the glasshouse assays and other selected alternative products.

Five replicated outdoor trials compared the myrtle rust efficacy of 16 different alternative products. Each trial used natural infection and 4–6 repeated applications of each product in a protective context, i.e. applications started before rust had become established in the trials. The alternative products were also compared with the synthetic fungicide triadimenol (Group 3 demethylation inhibitor) to provide a reference for their performance. The following were tested:

- ACVM-registered materials, including copper hydroxide, copper oxide, copper oxychloride, sulphur, lime sulphur, potassium silicate, potassium fatty acid soap and potassium bicarbonate. These could be used for myrtle rust control under off-label use conditions at appropriate application rates derived from the product labels and following any controls under their HSNO approval.
- ACVM-registered biological control agents (the *Bacillus subtilis* based products, Serenade® Optimum and Bacstar). These could also be used under off-label use conditions, as above.
- Non-ACVM-registered materials, including sodium bicarbonate (baking soda), sodium chloride (sea salt), two plant oil extracts (rosemary oil and aniseed oil) and two chitosan-derived polysaccharide bioactive fractions (PB-2 and PB-4). These could only be used on non-food plants, such as ornamentals, amenity trees and nursery plants not destined for food production. Approval to use these materials in the study, was obtained from the Environmental Protection Authority (EPA).

The main findings about myrtle rust control and plant damage (phytotoxicity) caused by the products in the outdoor trails were as follows:

- The synthetic fungicide (triadimenol), the three copper fungicides and sulphur at a 0.3% application rate all had high efficacy in controlling myrtle rust and posed negligible risk of phytotoxicity, although, at 0.6%, sulphur did tend to cause phytotoxicity. These results show that these products could all act as effective protectant fungicides in myrtle rust control programmes.
- The two chitosan products at 1% and 2%, rosemary oil at 1%, aniseed oil at 1% and the *Bacillus subtilis* biologicals, Bacstar and Serenade Optimum at label rates all had slight myrtle

rust efficacy and some risk of phytotoxicity at higher application rates. Bacstar appeared to perform slightly better than Serenade. These products could be useful in low disease risk situations, such as on more resistant hosts (e.g., mānuka seedlings) and more susceptible species during periods of low myrtle rust risk. e.g., between late autumn and early spring (May to September).

- Sodium bicarbonate at 1% and potassium bicarbonate at 0.5% (label rate) had poor activity against myrtle rust and often gave no better control than water-sprayed plants. At higher application rates they tended to control rust better but also caused greater phytotoxicity. These bicarbonate products cannot be expected to give reliable myrtle rust control, and their use could lead to plant damage.
- Potassium soap and potassium silicate at label rates gave negligible control of myrtle rust and caused phytotoxicity at higher rates. These appear to be unsuitable for use in controlling myrtle rust. Sea salt, which was included in one trial to test the hypothesis that sea salt spray inhibits myrtle rust infection in coastal areas, gave no myrtle rust control and caused severe phytotoxicity (growth suppression and shoot tip dieback) at the 1% rate used.

Further research is needed to develop and optimise formations suitable for spray application for the two chitosan products, rosemary oil and aniseed oil.

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1 General introduction

Myrtle rust caused by the fungal pathogen, *Austropuccinia psidii*, is a serious disease of Myrtaceous plants worldwide. The pathogen is native to South and Central America (Coutinho et al. 1998) and since the 1970s has been spreading in southern hemisphere Myrtaceae-dominated forests (Carnegie and Pegg 2018). It was first detected in New Zealand in 2017 and poses a major threat to multiple indigenous (taonga) and important economic species in New Zealand. The pathogen has been found on at least 23 host species, including native species (Chng et al. 2019) and the most at-risk native species are maire tawake (swamp maire; *Syzygium maire*), ramarama (*Lophomyrtus bullata*), rōhutu (*L. obcordata*), hybrids between *L. bullata* and *L. obcordata* and pōhutukawa (*Metrosideros excelsa*), particularly pōhutukawa seedlings (Beresford & Wright 2022).

Native Myrtaceae have significant cultural and ecological importance to New Zealand and, along with exotic Myrtaceae, also support many of the country's plant-based economies such as honey, essential oils, forestry, horticulture, plant propagation, floriculture and tourism industries. Myrtle rust is seen as a significant threat to both indigenous ecosystems and plant industries and there is concern that some taonga species may be susceptible to localised extinction. Chemicals have long been used to control pests and diseases in agriculture and fungicides form a significant component of disease management programmes for yield-driven crops. Myrtle rust can be effectively controlled on New Zealand native Myrtaceae using conventional synthetic fungicides (Beresford & Wright 2022). However, public concerns over effects of synthetic fungicides on human health and on the environment have underpinned an urgent need to identify alternative control options (Chock et al. 2020) and products that are effective and safe to use (Pérez-Pizá et al. 2024).

There is increasing interest in alternative control options including 'soft' chemicals and biologically based control methods for plant disease management (Scortichini 2022; McLaughlin et al. 2023). A review of alternatives for rust management by Chng et al (2019) identified plant essential oils and chemicals, including sodium bicarbonate (baking soda) and sulphur, as potential candidates to control myrtle rust. The aim of this project was to perform a series of experiments to determine the efficacy of selected alternative products, and a variety of ACVM-registered commercial fungicides, to control myrtle rust in two susceptible Myrtaceous species, *Lophomyrtus* sp. 'Red Dragon' and pōhutakawa.

Experiments were conducted on potted plants in the Ministry for Primary Industries (MPI) approved containment glasshouse facilities at The New Zealand Institute for Plant and Food Research Institute (PFR), Ruakura (#16609), and PFR, Lincoln (#16601), and at an outdoor site at PFR, Pukekohe. The glasshouse experiments enabled robust evaluation of non-registered control agents under environmental conditions that were conducive for myrtle rust infection. Experiments at Ruakura focused on biological control agents, plant-derived oils and plant defence-inducers whilst studies at Lincoln focused on novel bacterial antagonists sourced from native myrtaceous species. The outdoor experiments at Pukekohe enabled evaluation and optimisation of readily available ACVM registered products under natural conditions. This site also provided a pathway for more rigorous assessment of any new control agents identified in the glasshouse assays (subject to Environmental Protection Authority (EPA) approvals).

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2 Ruakura glasshouse assays

2.1 Introduction

Recent research by PFR in the Ministry of Business, Innovation and Employment (MBIE)-funded study 'Beyond myrtle rust' (programme # C09X1806), evaluated various natural products, biological control agents (BCAs), and inducers of plant defence for their potential to control myrtle rust on *Lophomyrtus* sp 'Red Dragon' and *Metrosideros excelsa* (pōhutukawa). Experiments were conducted under controlled conditions on potted plants in quarantine glasshouses. Candidates that achieved greater that 30% efficacy, including the registered bio-fungicide, Bacstar®, cinnamic acid, sodium salicylate, aniseed oil, rosemary oil and lemongrass oil were selected for further evaluation in this study. Various essential oils have demonstrated activity against plant pathogens including rust fungi (Santiago-Santiago et al. 2023; Marin et al. 2024) whilst salicylic acid and cinnamic acid are known to play key roles in plant defence against pathogen attack (Yang et al. 2023; Klessig et al. 2018).

Additional candidates, including sodium bicarbonate, potassium bicarbonate and chitosan were selected based on results from related PFR-funded studies and from the literature. Sodium and potassium bicarbonate have been long been proposed to have antifungal activity and to offer potential efficacy against rust fungi (Arslan et al. 2006; Azmeraw et al. 2020). Chitosan is a naturally occurring polysaccharide found in various fungi, insects and crustaceans (Román-Doval et al. 2023). Chitosan polymers can exhibit antimicrobial and/or plant defence inducing capability and there is increasing interest in the use of chitosan-based formulations for plant disease control world-wide (Riseh et al. 2022).

2.2 Materials and methods

2.2.1 Treatments

The range of control agents tested in this study are listed in Table 1.

Table 1. Control agents evaluated in glasshouse assays at PFR Ruakura between November 2023 and February 2025.

Treatment	Supplier	Mode of Action	Active ingredient content	Registered in NZ
Bacstar®	UPL Ltd	Biological control agent	*Bacillus subtilis* var. *amyloliquefaciens* D747	BioGro Certified
Aniseed oil	The Essential oils of NZ Ltd	Antimicrobial	Aniseed oil	No
Rosemary oil	The Essential oils of NZ Ltd	Antimicrobial	Rosemary oil	No
Lemongrass oil	The Essential oils of NZ Ltd	Antimicrobial	Lemongrass oil	No
Seaweed Organic Plant Tonic	Tui Products Ltd	Antimicrobial	Seaweed extracts	BioGro Certified
Conqueror spraying oil	Yates NZ	Antimicrobial	Mineral oil	BioGro Certified
Sodium bicarbonate	Sigma-Aldrich	Antimicrobial	Sodium bicarbonate	No
Potassium bicarbonate	Sigma-Aldrich	Antimicrobial	Potassium bicarbonate	No
Chitosan PB1	PFR*	Antimicrobial	Chitosan hydrochloride	No
Chitosan PB2	PFR*	Antimicrobial	Chitosan oligosaccharide	No

Treatment	Supplier	Mode of Action	Active ingredient content	Registered in NZ
Chitosan PB3	PFR*	Antimicrobial	Chitosan hydrochloride	No
Chitosan PB4	PFR*	Antimicrobial	Chitosan oligosaccharide	No
Cinnamic acid	Sigma-Aldrich	Plant defence inducer	Cinnamic acid	No
Sodium salicylate	Sigma-Aldrich	Plant defence inducer	Sodium salicylate	No
Kiwicare® Organic Super Sulphur	Kiwicare	Fungicide	Sulphur	BioGro Certified

* PFR funded project Bioactive polysaccharides (SSER D1823)

2.2.2 Potted plants

Glasshouse assays were conducted on potted *Lophomyrtus* sp. 'Red Dragon' (*L .bullata* x *L. obcordata* natural hybrid) and *Metrosideros excelsa* (pōhutukawa) (Figure 1), hereafter referred to as lophomyrtus and pōhutukawa, respectively.

Figure 1. Potted lophomyrtus (left) and pōhutukawa (right) plants as used in glasshouse experiments.

Prior to each assay, plants were graded by size (15–25 cm tall) and examined to ensure that each plant had healthy new growth that would be susceptible to inoculation with *A. psidii*. The plants were then arranged into groups comprising 10 replicate plants per treatment. Treatments were prepared in sterile reverse osmosis (RO) water containing a wetting agent (0.01% Tween 20, Sigma-Aldrich or 0.05% Du-Wett, UPL Ltd, NZ) and were applied to run-off using 500-mL hand-held trigger spray bottle in a designated secure spray area. Application timing, relative to pathogen inoculation, depended on primary mode of action and/or label recommendations; plant defence inducers were applied between 5–7 days before inoculation and antimicrobials were applied 1 day before pathogen inoculation.
A positive control (Bacstar®, a registered bio-fungicide for control of various fungi including rust on turf) was included in each assay and was applied 3 days before inoculation as per label instructions.

2.2.3 Pathogen inoculation

Treated plants were transferred to a containment glasshouse facility (#16609) for inoculation with *A. psidii*. The inoculum was prepared from a stock supply of *A. psidii* urediniospores that had been maintained at -80°C. Inoculum viability was checked prior to each assay by recording germination frequency after overnight incubation on water agar incubated at 20°C in the dark. Inoculum was prepared by suspending *A. psidii* urediniospores in Pegasol™ mineral oil at a concentration of 1 mg/mL, equal to approximately 5×10^5 urediniospores per ml. Plants were inoculated using a hand-held 100-mL capacity airbrush sprayer (Badger Air-Brush Co. US) set at 40 psi to achieve a fine mist of droplets over the plants. Immediately after inoculation, plants were placed on a moistened capillary mat in high humidity tents (>90% relative humidity (RH)) and arranged in a randomised complete block design. After 48 h, small vents in the humidity tents were opened to reduce RH to between 60–70%. Symptoms of myrtle rust infection on treated and untreated plants in each experiment were assessed at 14-21 days after inoculation and scored using a myrtle rust severity index that was developed for lophomyrtus and pōhutukawa (Table 2, Figure 2).

Table 2. Visual disease severity index (0 to 5 scale) for myrtle rust on glasshouse potted plants of lophomyrtus and pōhutukawa.

Index score	Symptom
0	No sporulation
1	Minor, 1-2 small pustules on leaf or stem and/or hypersensitive response brown spots
2	Minor/moderate - 2-3 pustules on stems and leaves
3	Moderate - 10-15 pustules on stems and leaves
4	Moderate/severe - sporulation on most areas - leaves, stem, and auxiliaries, 50-70% covered in pustules.
5	Severe - sporulation on all areas – leaves, stem, and auxiliaries, >75% covered

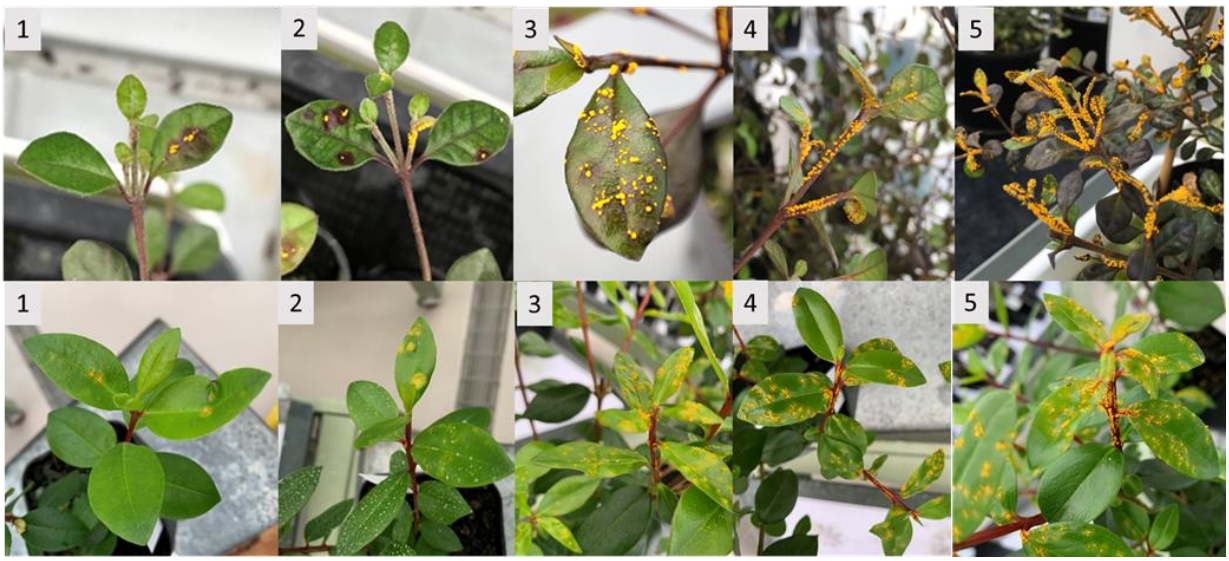

Figure 2. Examples of severity index symptoms on lophomyrtus (top row) and pōhutukawa (bottom row) in glasshouse experiments.

2.2.4 Statistical analyses

Mean scores were calculated per plant, and these were then used to calculate treatment means and standard errors. Differences between the treatments were tested using linear mixed effects model, with fixed effect for treatment and random effects for replicate and plant within replicate. Each trial was analysed separately, and the lophomyrtus and pōhutukawa results were analysed separately. Where there was a significant treatment effect, pairwise comparisons between treatments were carried out using Tukey's test. The R procedures lmerTest (Kuznetsova et al. 2020) and emmeans (Lenth et al. 2024) were used. As a check, the scores were also analysed as ordered categories, following Bürkner and Vuorre (2019). Medians and inter-quartile ranges were calculated (see excel data sheet). Similar effects were fitted (fixed effect for treatment, random effect for plant), and each trial was analysed separately. Treatment effects and credible intervals were inspected to determine which treatments differed from each other. The conclusions were very similar to the linear mixed effects models.

2.3 Results

A series of glasshouse assays was conducted to compare the efficacy of selected treatments against myrtle rust under controlled conditions.

2.3.1 Essential oils, mineral oil and seaweed extract

Lophomyrtus

- Bacstar, the positive control, was the most effective treatment and reduced myrtle rust by 41% in assay 1 and 66% in assay 2, compared with the untreated control (Table 3).
- The essential oils and Conqueror spraying oil reduced myrtle rust by between 20 and 29% in assay 1.
- Seaweed organic tonic was not effective ($p>0.05$) in assay 1 (16% efficacy) but did reduce disease significantly (24%) in assay 2.
- In assay 2, Conqueror had significantly ($p<0.05$) greater efficacy (35%) than rosemary oil (21%) but not aniseed oil (24%), lemongrass oil (32%) or seaweed organic tonic (24%).

Pōhutukawa

- Only Bacstar (27% efficacy) and lemongrass oil (23%) provided a significant control of myrtle rust on pōhutukawa.
- Bacstar was less effective on pōhutukawa compared with lophomyrtus.

Table 3. The efficacy of essential oils, mineral oil and seaweed extract on myrtle rust severity compared with Bacstar® (positive control) and the untreated (wetter only) on potted lophomyrtus and pōhutukawa plants. Assay 1 was conducted in January 2024 and assay 2 in March 2024. In each case disease was assessed 18 days after inoculation with *Austropuccinia psidii* urediniospores.

Treatment	Application rate (ml or g/L)	Timing (days before inoculation)	Assay 1				Assay 2	
			Lophomyrtus		Pōhutukawa		Lophomyrtus	
			Disease score	% Efficacy	Disease score	% Efficacy	Disease score	% Efficacy
Wetter	-	1 d	4.03 a	-	2.86 a	-	3.92 a	-
Bacstar®	1.5 g	3 d	2.37 c	41	2.13 b	26	1.34 d	66
Rosemary oil	20 mL	1 d	2.98 bc	26	2.24 ab	22	3.07 b	22
Aniseed oil	20 mL%	1 d	3.22 b	20	2.36 ab	17	2.98 bc	24
Lemongrass oil	10 mL%	1 d	3.02 bc	25	2.19 b	23	2.66 bc	32
Seaweed organic tonic	2.2 mL	1 d	3.39 ab	16	2.30 ab	20	2.97 bc	24
Conqueror spraying oil	10 mL	1 d	2.87 bc	29	2.40 ab	16	2.56 c	35

All treatments prepared in water containing 0.01% Tween 20 (Wetter). Values in each column with the same letter are not statistically different (p<0.05). Efficacy was calculated as the % reduction in disease on treated plants compared with the wetter according to the following formula: $\frac{W-T}{W}\ x\ 100$
where W = wetter and T = treatment.

Lophomyrtus

A third assay was performed with essential oils on lophomyrtus plants to compare effects of pre- and post-inoculation spray on disease control (Table 4). A water-soluble chitosan-oligosaccharide (PB-2) was included after obtaining evidence of activity against myrtle rust in a PFR-funded project (SSER-D1823).

- In this assay, the positive control, Bacstar reduced myrtle rust by 41% compared with the wetter-only control.
- The essential oils reduced myrtle rust by 19–22% when applied 1 day before inoculation and by 13-18% when applied 1 day after inoculation.
- The most effective treatment was chitosan oligosaccharide PB-2 which reduced disease by 50% when applied 1 day before inoculation but by only 13% when applied 1 day post-inoculation. This suggests good protectant properties but no significant reach-back activity.
- The activity of chitosan PB-2 was considered sufficient, when applied before inoculation to justify further investigation (see Section 1.2.3).

Table 4. Effects of pre- and post-inoculation applications of biofungicides on myrtle rust severity (0 to 5 scale) on potted lophomyrtus plants. Plants were inoculated with *Austropuccinia psidii* urediniospores on 11 April 2024 and disease was recorded 18 days later.

Treatment	Application rate (mL or g /L)	Timing Days before or after inoculation	Rust severity score	Efficacy (%)
Wetter			4.14 a	0
Bacstar®	1.5 g	3 d before	2.46 c	41
Rosemary oil	20 mL	1 d before	3.36 b	19
Aniseed oil	20 mL		3.32 b	20
Lemongrass oil	10 mL		3.22 b	22
Chitosan PB-2	10 g		2.08 c	50
Rosemary oil	20 mL	1 d after	3.60 b	13
Aniseed oil	20 mL		3.40 b	18
Lemongrass oil	10 mL		3.52 b	15
Chitosan PB-2	10 g		3.60 b	13

All treatments prepared in water containing 0.01% Tween 20 (Wetter). Values in each column with the same letter are not statistically different ($p<0.05$). Efficacy was calculated as the % reduction in disease on treated plants compared with the wetter according to the following formula:
$\frac{W-T}{W} x\ 100$
where W = wetter and T = treatment.

2.3.2 Antifungals and plant defence inducers

The activities of sodium bicarbonate, potassium bicarbonate, salicylic acid and cinnamic acid were compared on potted lophomyrtus and pōhutukawa plants (Table 5).

Lophomyrtus

- All treatments caused a significant reduction ($p<0.05$) in rust infection compared with the wetter control.
- The positive control, Bacstar, was the most effective treatment and reduced myrtle rust severity by 68%.
- The other treatments reduced rust severity by between 28% and 36%.

Pōhutukawa

- None of the treatments resulted in a significant reduction ($p>0.05$) in rust infection.

Table 5. Effects of antifungals and plant defence inducers on myrtle rust infection in lophomyrtus and pōhutukawa. Plants were inoculated with *Austropuccinia psidii* urediniospores on 18 January 2024 and disease was recorded 20 days later.

Treatment	Application rate (g/L)	Timing (days before inoculation)	Lophomyrtus		Pōhutukawa	
			Disease score	% Efficacy	Disease score	% Efficacy
Wetter		1	3.94 a		2.39 a	
Bacstar®	1.5	3	1.25 c	68	1.76 a	ns
Cinnamic acid	1.0	7	2.85 b	28	2.01 a	ns
Sodium bicarbonate	20	1	2.53 b	36	1.66 a	ns
Potassium bicarbonate	20	1	2.85 b	28	2.01 a	ns
Sodium salicylate	1.0	7	2.75 b	30	1.92 a	ns

All treatments prepared in water containing 0.05% Du-Wett (Wetter). Values in each column with the same letter are not statistically different ($p<0.05$). Efficacy was calculated as the % reduction in disease on treated plants compared with the wetter according to the following formula: $\frac{W-T}{W}\ x\ 100$ where W = wetter and T = treatment.

2.3.3 Chitosan

Chitosan is a naturally occurring polysaccharide and its solubility and bioactivity depend on its molecular structure (e.g. polymer chain length, degree of acetylation) (Román-Doval et al. 2023). Four chitosan fractions were evaluated for activity against myrtle rust, two oligosaccharide fractions (PB-2 & PB-4) with molecular mass <3 kilo daltons (kDa) and two chitosan hydrochloride fractions (PB1 & PB-3) with molecular mass<20 kDa (Table 6).

Table 6. Effect of different chitosan fractions on myrtle rust severity on lophomyrtus potted plants in glasshouse studies. Plants were inoculated with *Austropuccinia psidii* urediniospores on 20 May 2024 and disease was recorded 19 days later.

Treatment	Application rate (g/L)	Application timing	Rust severity score	Efficacy (%)
Wetter			3.40 a	
Bacstar®	1.5	3 d before inoculation	1.13 c	67
PB-1	10	5 d before inoculation	1.82 b	46
PB-2	10		1.97 b	42
PB-1+2	10 +10		1.90 b	44
PB-3	10		1.97 b	42
PB-4	10		2.17 b	36
PB-3+4	10 + 10		1.84 b	46

All treatments were prepared in water containing 0.05% Du-Wett (Wetter). Values in each column with the same letter are not statistically different ($p<0.05$). Efficacy was calculated as the % reduction in disease on treated plants compared with the wetter according to the following formula: $\frac{W-T}{W}\ x\ 100$ where W = wetter and T = treatment.

- The positive control, Bacstar, was the most effective treatment and reduced myrtle rust severity by 67%
- All of the chitosan extracts significantly reduced myrtle rust severity ($p<0.05$) compared with the control
- Overall, the efficacy of the chitosan fractions ranged from 36–46%.

The efficacy of PB2 and PB4 were compared on lophomyrtus and pōhutukawa potted plants with the commercially available sulphur-based product Kiwicare Organic Super Sulphur (Table 7). The chitosan fractions were applied twice, at 5 days and 1 day before inoculation to accommodate potential dual activity with the longer interval to allow time for plant defence induction and the 1-day interval for protectant antifungal activity.

Table 7. Effects of PB2, PB-4 and Kiwicare Organic Super Sulphur against myrtle rust on lophomyrtus and pōhutukawa potted plants. The assay on lophomyrtus was conducted in August 2024 whilst that on pōhutukawa was in October 2024. In each case disease severity was recorded 19 days after inoculation with *Austropuccinia psidii* urediniospores.

Treatment	Application rate (g/L)	Application timing; days before inoculation	Lophomyrtus		Pōhutukawa	
			Rust severity	Efficacy (%)	Rust severity	Efficacy (%)
Wetter			2.98 a		3.04 a	
Bacstar®	1.5	3	0.68 bc	77%	2.21 b	27%
Kiwicare Organic Super Sulphur	3.0	3	0.10 c	97%	1.26 c	59%
Chitosan PB-2	10	5 & 1	1.08 b	64%	1.78 bc	42%
Chitosan PB-4	10	5 & 1	1.11 b	63%	2.18 b	28%
Chitosan PB2+PB4	10 (each component)	5 & 1	0.6 bc	80%	1.87 bc	38%

All treatments prepared in water containing 0.05% Du-Wett (Wetter). Values in each column with the same letter are not statistically different (p<0.05). Efficacy was calculated as the % reduction in disease on treated plants compared with the wetter according to the following formula: $\frac{W-T}{W} x\ 100$ where W = wetter and T = treatment.

Lophomyrtus

- Bacstar significantly reduced (p<0.05) myrtle rust severity (77% efficacy) compared with the wetter-only control
- The most effective treatment, Kiwicare Organic Super Sulphur, reduced myrtle rust by 97%
- The chitosan fractions PB-2 and PB-4 reduced myrtle rust severity by 63–64% when used individually and by 80% when combined.

Pōhutukawa

- Bacstar reduced (p<0.05) myrtle rust severity by 27% efficacy compared with the wetter-only control
- Kiwicare Organic Super Sulphur was the most effective treatment and reduced myrtle rust severity by 59%
- The chitosan fractions PB-2 and PB-4 reduced myrtle rust severity by 42% and 28%, respectively, when used individually and by 38% when combined.

A follow-up experiment was conducted to determine whether there was any measurable benefit with combining two different chitosan fractions for myrtle rust control (Table 8). This was based on the hypothesis that the two oligosaccharides may have different modes of action that may provide additive control.

Table 8. Effects of PB2, PB-4, alone and combined, against myrtle rust on lophomyrtus potted plants. Plants were inoculated with *Austropuccinia psidii* urediniospores on 21 November 2024 and disease was recorded 19 days later.

Treatment	Application rate (g/L)	Application timing; days before inoculation	Lophomyrtus	
			Rust severity	Efficacy (%)
Wetter			4.30 a	
Bacstar®	1.5	3	2.43 b	43
PB-2	10	6 & 1	1.70 c	60
PB-2	20	6 & 1	1.29 cd	70
PB-4	10	6 & 1	1.58 cd	63
PB-4	20	6 & 1	1.24 cd	71
PB-2 + PB-4	10 + 10	6 & 1	1.14 d	73

All treatments prepared in water containing 0.05% Du-Wett (Wetter). Values in each column with the same letter are not statistically different (p<0.05). Efficacy was calculated as the % reduction in disease on treated plants compared with the wetter according to the following formula: $\frac{W-T}{W} x\ 100$ where W = wetter and T = treatment.

- All treatments significantly (p<0.05) reduced myrtle rust severity compared with the wetter-only-control
- All chitosan treatments were significantly more effective than Bacstar
- The combination of PB2 + PB4 had higher efficacy that the other treatments but did not differ significantly (p<0.05) compared with PB-2 (20 g/L) or PB-4 (10 or 20 g/L)
- The data suggest that efficacy is related to chitosan concentration and that there is no evidence of synergies between component parts.

An additional experiment was performed to compare the efficacy of PB-2 and PB-4 at 1% (v:v) versus 0.25% (v:v) (Table 9). This experiment also compared the efficacy of organic super sulphur when applied pre-inoculation and post-inoculation.

Table 9. Effects of PB2, PB-4, alone and Kiwicare Organic Super Sulphur, against myrtle rust on lophomyrtus potted plants. Plants were inoculated with *Austropuccinia psidii* urediniospores on 31 January 2025 and disease was recorded 20 days later.

Treatment	Application rate (g/L)	Application timing; days before or after inoculation	Lophomyrtus	
			Rust severity	Efficacy (%)
Wetter			3.82 a	
Bacstar®	1.5	3 d before	2.13 c	44
PB-2	2.5	6 & 1 d before	2.08 c	46
PB-2	10	6 & 1 d before	1.39 b	64
PB-4	2.5	6 & 1 d before	1.98 c	48
PB-4	10	6 & 1 d before	1.30 b	66
Kiwicare Organic Super sulphur	3	3 d before	0.20 e	95
Kiwicare Organic Super Sulphur	3	3 d after	3.0 b	21

All treatments prepared in water containing 0.05% Du-Wett (Wetter). Values in each column with the same letter are not statistically different (p<0.05). Efficacy was calculated as the % reduction in disease on treated plants compared with the wetter according to the following formula: $\frac{W-T}{W} x\ 100$ where W = wetter and T = treatment.

- All treatments significantly ($p<0.05$) reduced myrtle rust severity compared with the wetter-only control
- The most effective treatment, Kiwicare Organic Super Sulphur, reduced disease severity by 95% when applied 3 days before inoculation, but by only 21% when applied 3 days after inoculation
- This indicates that sulphur primarily functions as a protectant
- Chitosan PB-2 and PB-4 showed similar levels of efficacy to each other and reduced disease severity by ~65% at 10 g/L and by ~47% when applied at 2.5 g/L
- Chitosan efficacy is concentration dependent.

2.4 Conclusions

Fifteen different agents were evaluated for their potential to protect lophomyrtus and pōhutukawa potted plants against myrtle rust. The microbial antagonist, Bacstar, selected as a positive control based on earlier efficacy data, reduced myrtle rust severity on lophomyrtus plants by between 41–77% across nine experiments. The efficacy of Bacstar on pōhutukawa was lower than on lophomyrtus, however, the same was true for each of the control agents tested. The reason for the difference in efficacy between the two Myrtaceae is unknown and is beyond the scope of this study. However, myrtle rust severity was generally lower on pōhutukawa than lophomyrtus, but this alone is unlikely to explain the difference in efficacy. Nevertheless, the results indicate that efficacy data should not be extrapolated from an individual species and that the activity of any single agent against myrtle rust may differ across myrtaceous species. This is an important consideration when evaluating any ‘new’ agent for myrtle rust management.

The most effective treatment in the glasshouse trials was Kiwicare Organic Super Sulphur, a registered product that is available from garden centres and supermarkets. This sulphur product reduced myrtle rust severity by ~96% on lophomyrtus and by ~59% on pōhutukawa when applied before pathogen inoculation. A follow-up study showed that efficacy, when applied after pathogen inoculation, the efficacy fell to 21% on lophomyrtus suggesting that sulphur provides little curative activity and primarily functions as a protectant. The same product was further evaluated in outdoor potted plants trials (see Section 4)

Of the ‘alternative’ control agents tested against myrtle rust, the greatest promise was shown by water-soluble chitosan. Four different chitosan oligosaccharides performed consistently against myrtle rust on lophomyrtus with efficacy ranging between 36–80% depending on source material and concentration. Preliminary evidence indicates that chitosan functions as a protectant and has no significant reach back activity, but further studies are necessary to confirm its mode of action. Nevertheless, chitosan efficacy was considered sufficient for further investigation and appropriate EPA approvals were obtained to enable inclusion of PB-2 and PB-4 in outdoor potted plant trials at Pukekohe (see Section 4).

The essential oils, aniseed oil, lemongrass oil and rosemary oil at concentrations of 1–2% (v:v) reduced myrtle rust by 20–30% on average on lophomyrtus and pōhutukawa. Similarly, the antifungal chemicals, sodium bicarbonate and potassium bicarbonate, and defence inducers, cinnamic acid and salicylic acid reduced myrtle rust by 28–38% on lophomyrtus but had no significant effect on myrtle rust severity on pōhutukawa. Of these, aniseed oil, rosemary oil, sodium bicarbonate and potassium bicarbonate were further tested in outdoor potted plant trials (see Section 4).

These glasshouse studies indicate that ‘alternative’ agents have potential to contribute to the management of myrtle rust. However, none are likely to equal the efficacy of a conventional synthetic fungicide when used as a standalone treatment. Instead, it may be necessary to develop integrated management programmes comprising combinations of biologicals, antimicrobials and plant defence inducers. Further studies are recommended to optimise control of myrtle rust through the integrated use of compatible and complementary treatment combinations.

2.5 Recommendations from glasshouse studies

Glasshouse assays have demonstrated to provide a robust and reliable option for the evaluation of ‘alternative’ control agents for myrtle rust under controlled conditions. However, differences in efficacy observed on lophomyrtus and pōhutukawa suggest that future studies should include at least three myrtaceous species to gauge potential variability.

Kiwicare Organic Super Sulphur was the most effective control agent identified in glasshouse assays. This is a registered product that is readily available in garden centres and supermarkets. Initial results indicate that it primarily functions as a protectant and that it has little reach-back activity. More detailed glasshouse studies are recommended to better understand its activity and to optimise application timing and frequency relative to pathogen infection.

Chitosan fractions show promise as potential ‘new’ control agents. Further evaluation of chitosan fractions is required to optimise efficacy through formulation development (i.e. blending fractions with different modes of action) and optimising of application timing to maximise both direct and indirect (plant defence induction) modes of action.

The essential oils, the bicarbonate salts and the defence inducers (cinnamic acid and salicylic acid) each exhibited low to moderate control activity but were likely not adequate to be recommended as stand-alone treatments. However, the combined or integrated use of antimicrobials, antagonists and/or plant defence inducers could be considered for control of myrtle rust. The potential benefits of combining agents with different modes of action to enhance disease control has been demonstrated in other crop systems (de Jong et al. 2019; Reglinski et al. 2023).

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3 Lincoln – Novel anti-microbial antagonists

3.1 Introduction

Plants contain numerous endophyte microorganisms that can have beneficial effects. Microbial endophytes have been shown to modulate plant growth, metabolite profile and disease resistance. Thus, endophyte communities imparted to myrtaceous seedlings through vertical or horizontal transmission may correlate with resistance status and provide a mechanism to support plant resistance. Previous studies using plant endophytes have shown promise for the control of coffee rust (da Silva et al. 2012; da Silva et al 2023), stripe rust of wheat (Kiani et al. 2021), white pine blister rust (Ganley et al. 2008) and myrtle rust (Chock et al. 2021).

During the MBIE-funded Endeavour Research Programme 'Beyond Myrtle Rust' a collection of over 2000 microorganisms (fungi and bacteria) was isolated from myrtaceous species, including endophytes and epiphytes. This collection was assessed for isolates with potential to antagonise the causal agent of myrtle rust, *A. psidii,* in a series of bioassays. From those experiments three candidate bacterial endophytes were identified with promising ability to inhibit myrtle rust (Table 10).

Table10. Endophyte isolates selected to assess as novel biocontrol agents against myrtle rust.

Strain #	Location of Origin	Host plant	Tissue of origin	Species
776	Bay of Plenty	*Metrosideros excelsa*	Mature stem	*Bacillus sp.*
881	Christchurch	*Leptospermum scoparium*	Mature leaves	*Pseudomonas sp.*
1067	Christchurch	*Leptospermum scoparium*	Mature stem	*Serratia sp.*

3.2 Materials and methods

3.2.1 Twiglet assay

Two experiments to optimise the application of candidate biocontrol agents were conducted using a detached axillary shoot assay (twiglet assay) to assess the three promising bacterial antagonists applied individually and in four different combinations. Three controls were included comprising the commercially available Bacstar as a positive control, in addition to the carrier control and a non-inoculated control (Table 11).

Young axillary shoots were harvested from clonally propagated *Lophomyrtus* sp. 'Red Dragon'. Each shoot, containing a minimum of two susceptible young leaves, was placed in a square Petri plate containing 1% water agar amended with benzylaminopurine. Each Petri plate contained three axillary shoots and was considered as one biological replicate. Each shoot was assessed independently.

Table 11. Treatments selected for *Lophomyrtus* 'Red Dragon' twiglet assay.

Treatment	Candidate microbial antagonists	Concentration cells/mL
1	Pseudomonas sp. strain 881	1 x 107
2	Bacillus sp. strain 776	1 x 107
3	Serratia sp. strain 1067	1 x 107
4	Pseudomonas sp. strain 881, Bacillus sp. strain 776	1 x 107 (50:50 v:v of each strain)
5	Pseudomonas sp. strain 881, Serratia sp. strain 1067	1 x 107 (50:50 v:v of each strain)
6	Bacillus sp. strain 776, Serratia sp. strain 1067	1 x 107 (50:50 v:v of each strain)
7	Pseudomonas sp. strain 881, Bacillus sp. strain 776, Serratia sp. strain 1067	1 x 107 (33:33:33 v:v of each strain)
8	Bacstar® (positive control, used at label rate of 1 g per L); Bacillus subtilis D747*	1 x 107 (equivalent to 1010 per L)
9	Carrier control (No microorganisms) / MR	-
10	Nil (Untreated control) No MR / No microorganisms	-

Each bacterial strain was cultured overnight in Luria broth (LB) medium. The culture suspensions were centrifuged to pellet the cells and the supernatant was discarded. The pellet was resuspended in saline solution (0.5% w/v NaCl) to achieve a final concentration of 10^7 cells per mL and applied to the axillary shoots by atomisation, 1 mL per Petri dish (three twiglets).

Austropuccinia psidii inoculum (90% germination), prepared at a concentration of 1 mg/mL spores suspended in carrier solution (0.9% NaCl / 1% Tween 20), was applied to the axillary shoots by atomisation.

Two experiments (experiment 1and experiment 2) were performed independently to assess the efficacy of the bacteria as curative (application after *A. psidii* inoculation) or protective (application before *A. psidii* inoculation) agents for myrtle rust, respectively.

3.2.1 EXPERIMENT 1_Curative

Petri plates each containing three plant shoots were inoculated with *A. psidii* and left covered in the) dark for 36 hours to allow rust spore germination and plant infection. After this period, shoots were inoculated with the bacterial treatments as described in Table 11 and plates were incubated horizontally for 12 additional days under natural photoperiod before evaluation. The control Petri plates, treatments 8 to 10, were treated as outlined in Table 11. There were nine replicate plates for each treatment.

3.2.2 EXPERIMENT 2_Protective

Petri plates each containing three plant shoots were inoculated with the bacterial treatments and controls, as described in Table 11, covered with the lid and incubated horizontally for 36 h on a bench in the glasshouse under natural photoperiod. For each treatment there were nine replicates. After this period, all shoots were inoculated with *A. psidii* and left covered in the dark for 36 h. Plates were then incubated for an additional 10 days under natural photoperiod.

Each experiment consisted of two independent replicate trials using the same experimental design. Shoot infection was scored 0–4 depending on severity of rust infection, where 0 is non-infected, 1 minor sporulation on leaves or stem, 2 moderate sporulation in leaves or stem, 3 severe sporulation on leaves or stems, and 4 dead leaves.

3.2.3 Glasshouse assay

Based on the results from the twiglet assays, which were ranked from lowest to highest efficacy against myrtle rust, the four most effective bacterial treatments were selected to apply to potted young plants in a glasshouse assay (Table 12). These treatments were applied only as curatives, as this had been shown to be the most effective timing of application.

Table 12. Treatments applied to potted plants of *Lophomyrtus* 'Red Dragon' in a glasshouse assay.

Treatment	Candidate microbial antagonists	Concentration cells/mL
1	Carrier control (Rust/No microorganisms)	NA
2	Rust only	NA
3	Bacstar® (positive control, used at label rate of 1 g per L); *Bacillus subtilis* D747*	5×10^{10} cfu/g
4	*Serratia* sp. strain 1067	1×10^{9}
5	*Bacillus* sp. strain 776	1×10^{9}
6	*Pseudomonas* sp. strain 881	1×10^{9}
7	Nil (Untreated control)	NA
8	*Pseudomonas* sp. strain 881 X *Serratia* sp. strain 1067	1×10^{9} (50:50 v:v of each strain)

Experiment was done from the 31/05/2024 to 17/06/2024.

Austropuccinia psidii spores were obtained from PFR, Ruakura and used to spray plants at a final concentration of 2 mg/mL due to lower germination rate (60%). Plants inoculated with A. psidii spores were left in the dark at room temperature for 3 days to allow spore germination and plant infection. After this period, bacterial inoculation was carried out by spraying the bacterial suspension onto the potted plants. Plants were then incubated for an additional 14 days at 18–23°C/16 h light/8 h dark photoperiod.

A randomised complete block design was used, with nine replicates per treatment. Disease was assessed using the symptom severity index described in Section 2.2, Table 2 (Ruakura).

To compare rust infection scores between treatments in twiglet and glasshouse experiments a generalized linear mixed effect (GLMM) model was performed in R (R Core Team 2022) using the brms package.

3.2.4 Microbiome analysis

The epiphytic (leaf surface) microbial communities (microbiome) from leaves of plants inoculated with *A. psidii* and bacterial strains from the potted plants experiment described in section 3.2.1 and 3.2.2

were analysed by metabarcoding. The purpose of this was to assess the establishment of the applied bacterial strains on the leaf surface. The two objectives for this part of the study were:

1. To assess changes to the composition of the leaf surface microbiome after rust infection
2. To assess the relative abundance of the applied bacterial strains on the leaf surface.

Samples were collected from individual treatments at the time points indicated in Figure 3. For all treatments three leaf samples were collected, each sample came from an individual plant (three plants per treatment). For each sample, three leaves from the same plant were pooled, washed by shaking in a solution of 0.9% NaCl/0.1% Tween 20 for 20 min. Leaves were discarded after washing and the liquid was centrifuged to collect the pellet. DNA was extracted from the pellet using the NucleoSpinPlant II Mini kit, Macherey-Nagel.

The microbiome analysis was done using the Illumina MiSeq platform (Sequench Ltd, Nelson). Two-step PCRs for DNA amplification were conducted using primers NSI1mod_MS/ Fun58A2R_MS to amplify fungi, and 1193r_MS/799F_MS to amplify bacteria. Both sets of primers included the MiSeq and Nextera adapter sequences MS. All the PCR products were purified using Agencourt AMPure XP PCR purification system (Agencourt Biosciences Corporation) before the sequencing process for amplicon analysis.

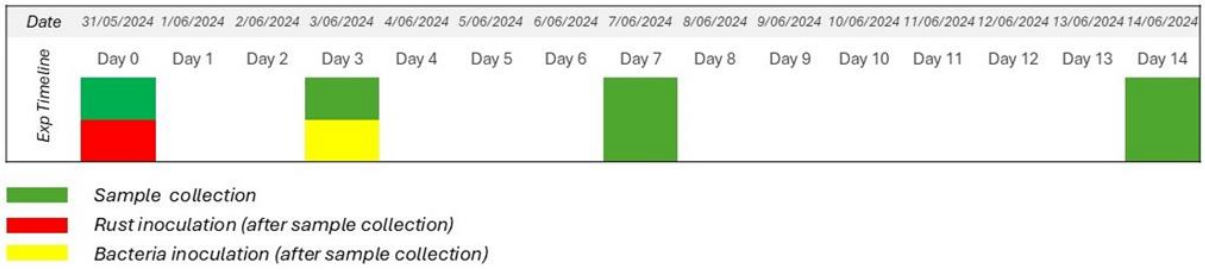

Figure 3. Timeline for *Austropuccinia psidii* infection, bacterial colonisation and sample collection for the microbiome analysis of leaves epiphytes.

3.3 Results

3.3.1 Twiglet assay

Based on a 95% credible interval, only the untreated control was significantly different in terms of average rust infection score for both experiments. The other treatments were not significantly different from each other but were ranked from highest to lowest severity score. Bacstar has the lowest infection severity score, and the carrier control had the highest infection severity score for both experiments (Figures 4 and 5).

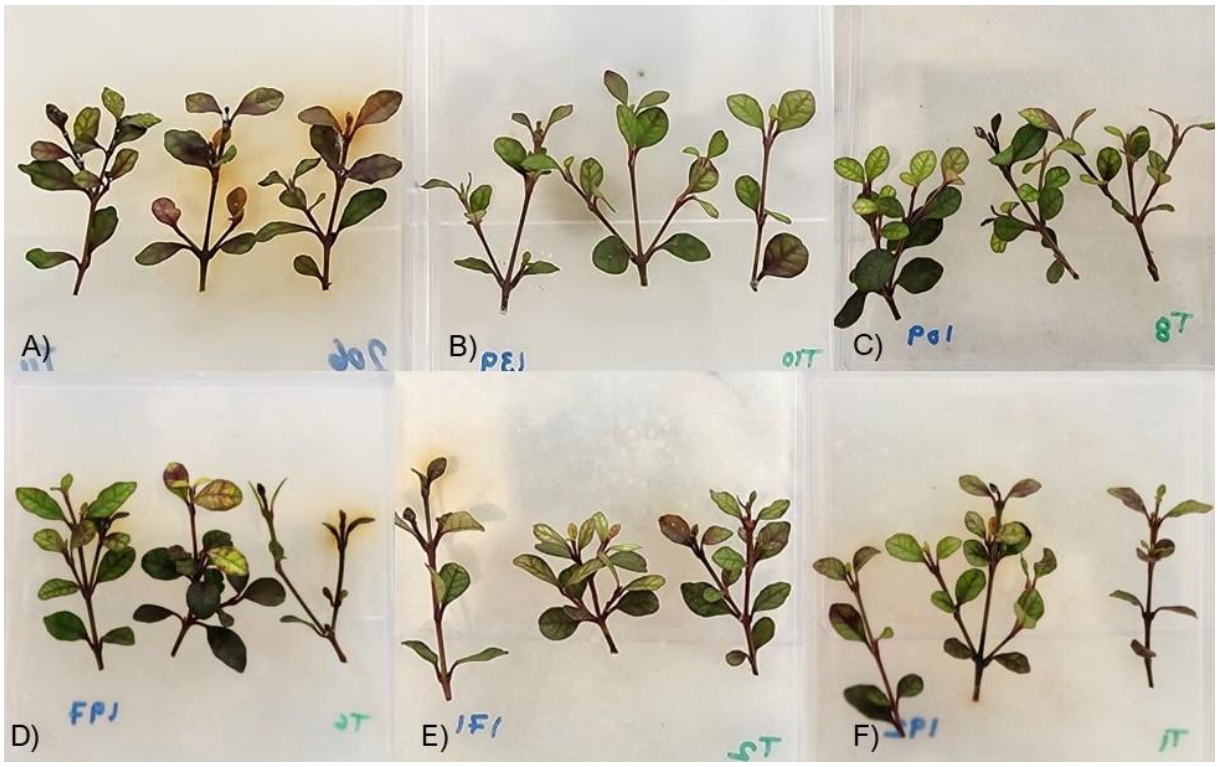

Figure 4. Some of the twiglet assay treatments for Experiment 1_Curative. A) rust only, B) Untreated plants, C) Bacstar®, D) *Serratia* sp. strain 1067 x *Bacillus* sp. strain 776, E) *Bacillus* sp. strain 776, F) *Pseudomonas* sp. strain 881.

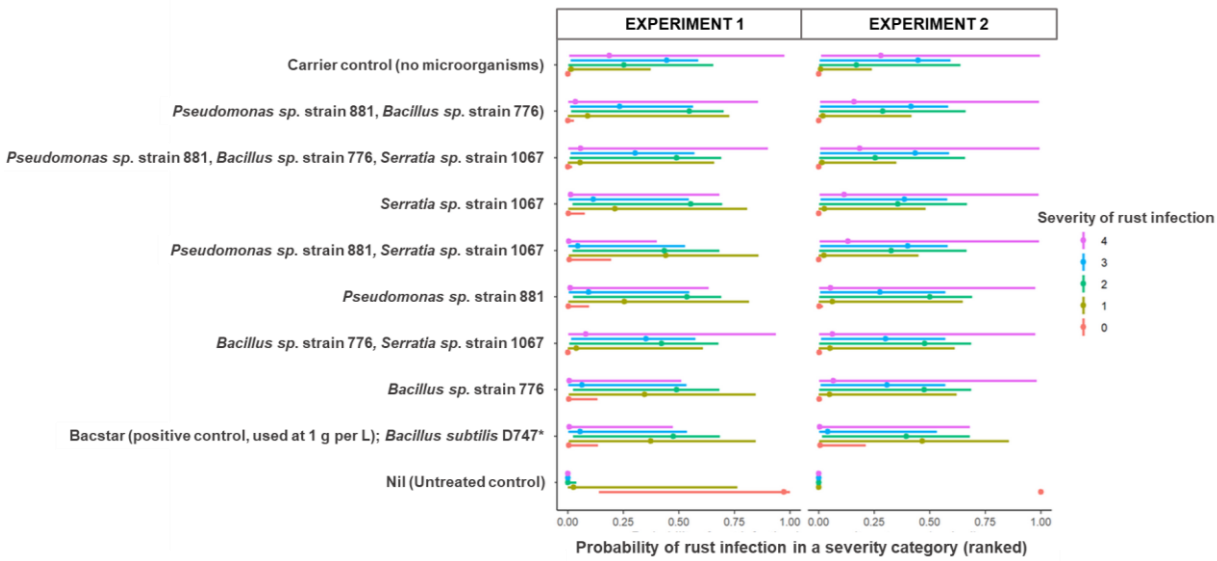

Figure 5. Conditional effect plot of the probability of rust infection in a severity category (0–4) for experiment 1 and experiment 2. Treatments are ranked from lowest infection severity (bottom) to highest infection severity (top). Displayed points are mean values and 95% credible intervals.

Results from the detached axillary shoots (twiglet assays) indicated that candidate bacterial antagonists were more effective against rust when applied as curative rather than protective treatments. According to Figure 6, where treatments were ranked from lowest to highest infection severity, the most effective treatments, apart from Bacstar, were *Bacillus* sp. strain 776, *Pseudomonas* sp. strain 881 and the combination of *Pseudomonas* sp. strain 881 + *Serratia* sp. strain 1067.

Based on the above observation from the greenhouse experiment, the treatments were selected for evaluation on potted *Lophomyrtus* sp. 'Red Dragon' plants.

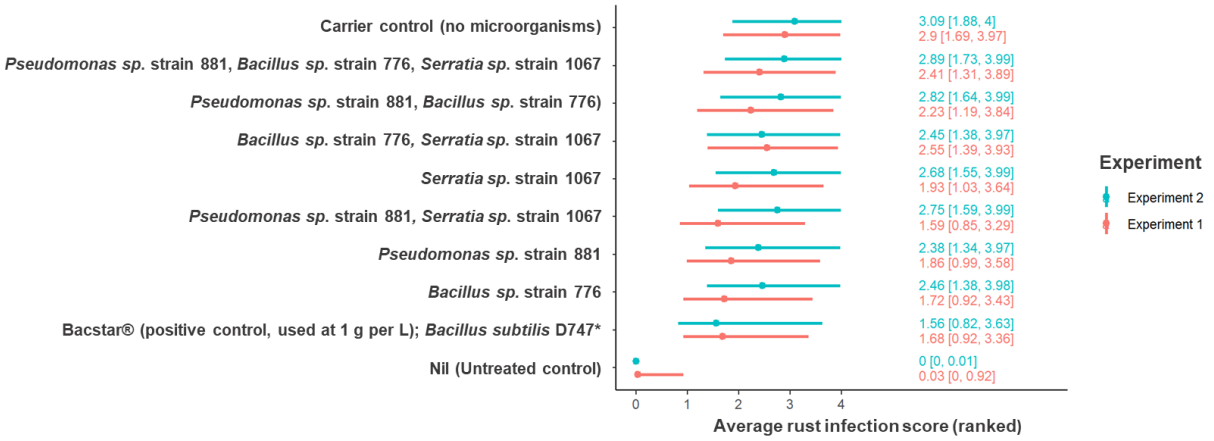

Figure 6. Conditional effect plot of the average rust infection score (0-4) for experiment one and experiment two. Treatments are ranked from lowest infection severity (bottom) to highest infection severity (top). Displayed points are mean values and 95% credible intervals with text alongside.

3.3.2 Greenhouse assay and microbiome analysis

Based on the average rust infection score from the two twiglet experiments, the treatments with the lowest infection score, *Bacillus* sp. strain 776, *Pseudomonas* sp. strain 881, *Serratia* sp. strain 1067 and a combination of *Pseudomonas* sp. strain 881 x *Serratia* sp. strain 1067 were selected to evaluate their curative activity on potted plants after inoculation with *A. psidii.* The bacterial antagonists were applied to the leaves 36 h after the plants were inoculated with *A. psidii*. The establishment of the microbiome on the leaf surfaces was assessed during the experiment using metabarcoding.

As shown in Figure 7, the plants treated with *Bacillus* sp. strain 776 had a significantly lower average rust infection score than the Bacstar positive control. *Pseudomonas* sp. strain 881 and *Serratia* sp. strain 1067 performed better in combination than individually, possibly having a synergistic effect. The results showed that *Bacillus* sp. strain 776 and the combination of *Pseudomonas* sp. strain 881 x *Serratia* sp. strain 1067 act as curative agents, reducing the rust infection once *A. psidii* has infected the plant. The positive effect of the bacteria is most evident in the new growth tissue of the plants (Figure 8, arrows).

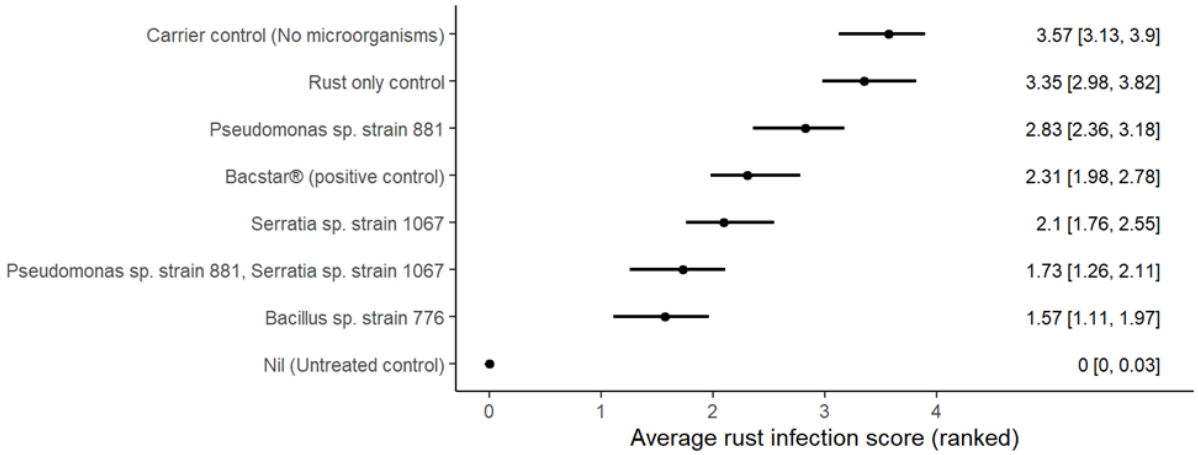

Figure 7. Effect of each treatment on the average rust infection score (ranked). The median value (points) and 95% credible interval (bars) are shown.

Figure 8. Rust infection severity on each treatment. A) Rust only, B) Bacstar® positive control, C) *Pseudomonas* sp. strain 881, D) *Bacillus* sp. strain 776, E) combination *Pseudomonas* sp. strain 881 x *Serratia* sp. strain 1067, and F) *Serratia* sp. strain 1067. Arrows shown new growth infection comparison between rust infected control (A) and best performing strains (D, E).

3.3.3 Leaf microbiome analysis

Changes to the relative abundance and composition of the 10 most abundant bacterial genera present on the leaf surface over time are shown in Figure 9. The bacterial community had changed in both composition and relative abundance three days after *A. psidii* inoculation, and before the bacterial inoculation. Inoculation with the selected bacteria after three days (Day 3) did not affect the relative composition of the bacterial community during the first four days after the inoculation with th bacteria (Day 7). This effect is likely to be the result of the spraying alone as it is also observed on the carrier control without rust. However, changes in the bacterial composition can be observed after another seven days (Day 14).

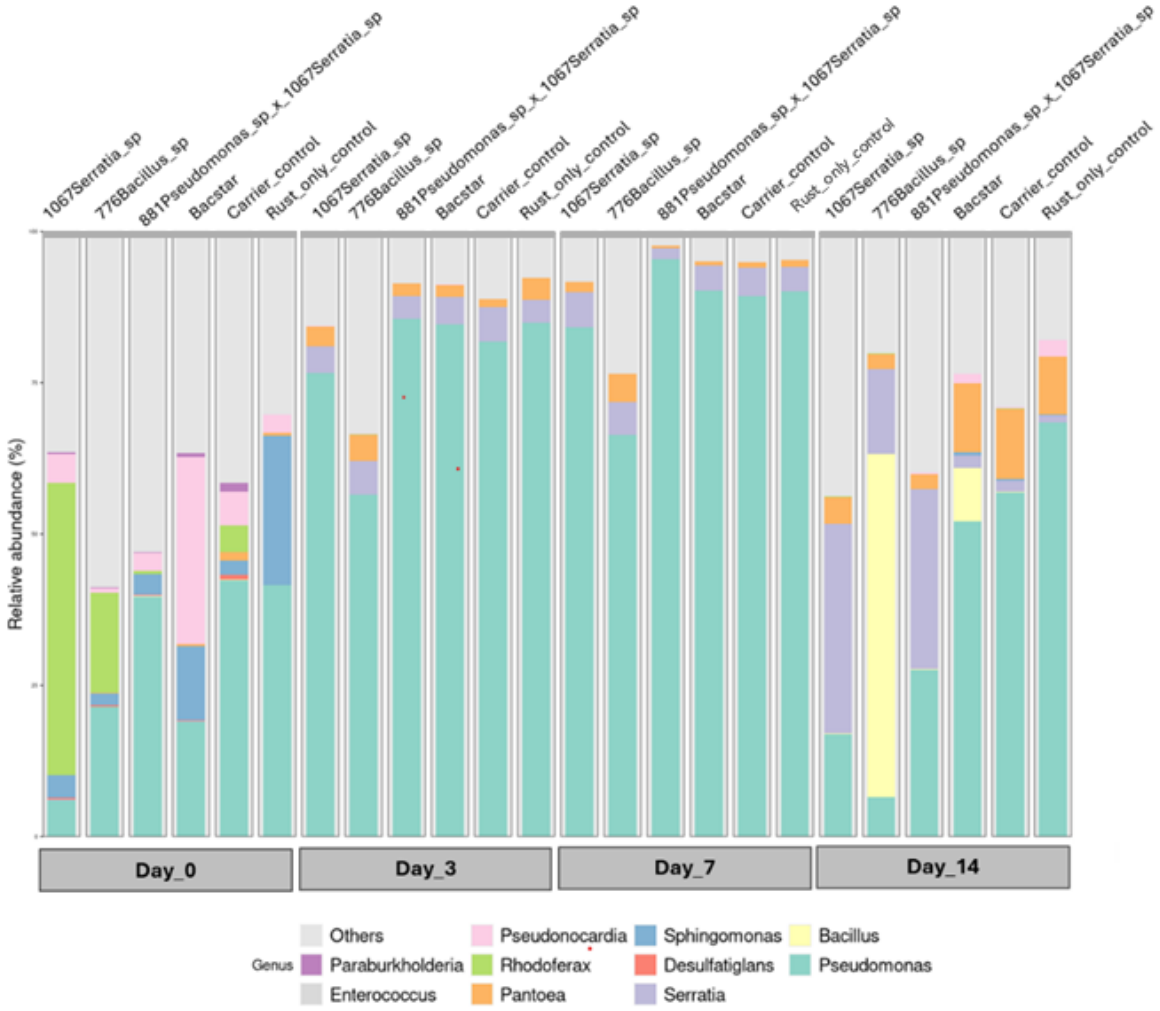

Figure 9. The relative abundance of the 10 most common genera of bacteria found on the leaf surface of treated plants.

An increase in the relative abundance of genera encompassing the applied biocontrol bacteria on the surface of the treated leaves can be observed 11 days after bacterial inoculation (Day 14), apart from treatment 8 (*Serratia* sp.strain 1067 x *Pseudomonas* sp. strain 881) where only the genus *Serratia* increased and not *Pseudomonas*. This may be related to the high relative abundance of *Pseudomonas* species present in this treatment from day 0 following spraying.

Greater relative abundance of the genus *Bacillus* in the isolate *Bacillus* sp. isolate 776 treatment at day 14 compared to the Bacstar (a *Bacillus* sp. commercial strain) treatment indicates different mode of action of the *Bacillus* strain in Bacstar, compared with our candidate strain. Our strain was more effective as a curative agent, which could be due to its capacity to survive and colonize the leaf surface more effectively.

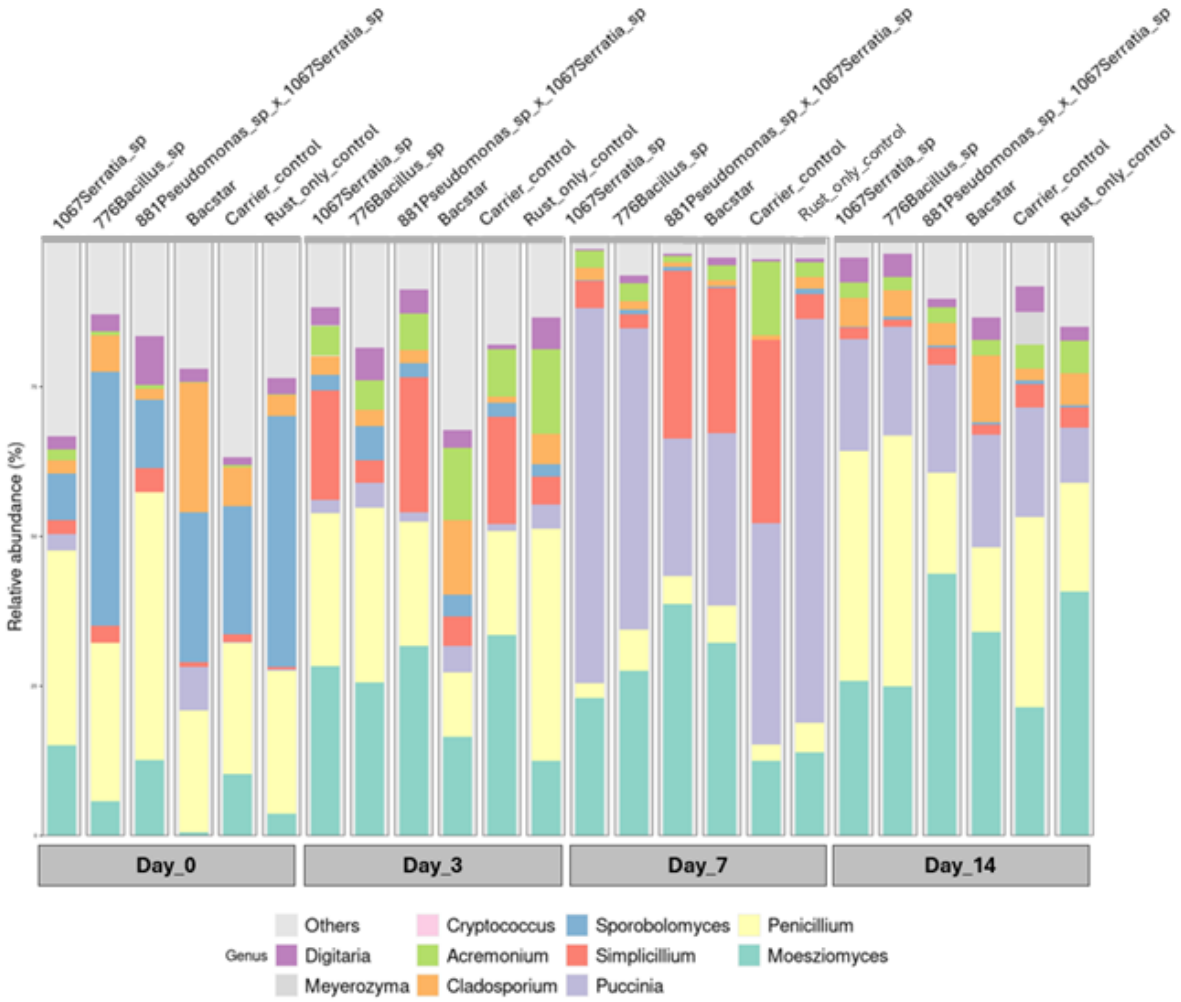

Figure 10. Fungal relative abundance observed on the leaf surface of treated plants. Table shows the 10 most abundant genera observed.

The fungal community was also analysed to monitor the establishment and dynamics of the rust population throughout the infection and biocontrol processes. Additionally, the fungal and broader microbial communities were examined to determine whether the presence of the rust and the application of the biocontrol bacteria induced significant changes in microbial composition, potentially influencing the infection and bioprotection outcomes.

According to our observations, the fungal community already present on the leaves was affected by the spraying process, with a reduction in the relative abundance of the genus *Sporobolomyces* from the original populations (Figure 10). As expected, an increase in the relative abundance of the rust (identified as *Puccinia* in Figure 10) was detected 7 days after inoculation in all treatments.

The genus *Puccinia* is considered to encompass myrtle rust because the reclassification of Puccinia *psidii* to *Austropuccinia psidii* (myrtle rust) is recent. The databases used for the analysis of the short DNA sequences (barcodes) amplified during metabarcoding still contain the species listed as *Puccinia psidii.*

Taking this taxonomic aspect into account, there are no changes observed in the relative abundance of the rust population after bacteria inoculation at any of the time points collected when compared with the controls, although the bacteria decreased disease. This may be because the leaves selected were those originally presenting rust infection symptoms, and not the healthy new growth.

Further analysis of the fungal community on the new growth of treated plants could clarify this observation.

3.4 Conclusions

Three promising bacterial strains, originally sourced as endophytes from myrtaceous species, were investigated for their ability to reduce disease caused by *A. psidii* on *Lophomyrtus '*Red Dragon', both as single species and as combinations. To guide the choice of treatments and timing for the potted plant assay, these candidate bacterial antagonists were first assessed for their ability to act as either protective and/or curative treatments in a twiglet assay. Ranking of the infection scores from the twiglet assay suggested that the candidate bacterial antagonists performed better as curative agents. In that assay, none of the candidate agents outperformed the Bacstar control. However, the twiglet assay was useful as a tool for rapid screening of multiple strain combinations.

Some of the candidate bacterial strains and combinations demonstrated the ability to inhibit *A. psidii* infection by ≥30% in the potted plant assays. Among the tested strains, *Bacillus* sp. strain 776 was the most effective, followed by the combination of *Pseudomonas* sp. strain 881 and *Serratia* sp. strain 1067. *Bacillus* sp. strain 776 was more effective than Bacstar in the potted plant assay. The mode of action of *Bacillus sp.* strain 776 appears to differ from the commercial product Bacstar (*Bacillus amyloliquefaciens*), as it exhibits a stronger curative effect, whereas Bacstar is more effective as a protective treatment. The degree of control observed, although exceeding Bacstar, is not approaching the efficacy of fungicide treatments. As Bacstar is a commercial product that is used on a range of crops, further taxonomic assessment of the candidate antagonistic *Bacillus* strain and its spectrum of activity in pathosystems in addition to myrtle rust is recommended.

Further assessment of the microbial dynamics on the leaf surface was carried out by metabarcoding and this was shown as a useful tool to assess relative abundance of bacterial genera. These results showed that *Pseudomonas* species initially had a high relative abundance on the leaves and this relative abundance increased in response to spraying across all treatments. This suggested that *Pseudomonas* species are common members of the *Lophomyrtus* 'Red Dragon' leaf microbiome and that they respond positively to leaf wetting and higher moisture availability. By day 14 the bacterial microbiome of the leaf had changed with the patterns reflective of the treatments applied. The relative abundance of the genera *Bacillus* and *Serratia* had increased on the leaf surface. The relative abundance of *Bacillus* in the *Bacillus* sp. strain 776 treatment was higher than in Bacstar-treated plants, and by day 14 *Bacillus* was only detected in treatments where it had been applied. From day 7 there were also increased relative abundance of rust fungi DNA on the leaf surface, aligning with myrtle rust symptom observations. Survival of the bacteria on the leaf surface is difficult due to the exposure to UV light and desiccation. The increased relative abundance of *Bacillus* in the *Bacillus* sp. 776 treatment suggests that this strain can colonise the phyllosphere and outcompete other bacteria

for that niche. However, absolute quantitation (e.g. quantitative polymerase chain reaction; qPCR) would be useful to confirm exact numbers on the leaf surface. Phyllosphere survival is a positive attribute for commercial strains.

The potted plant assay indicates that *Bacillus* sp. strain 776 has the potential to reduce the effects of myrtle rust on *Lophomyrtus* and that it can colonise the leaf surface for a sustained period. Although more effective than the current "best" commercial biocontrol agent, Bacstar, the control is not equivalent to the control exerted by conventional synthetic fungicides. To progress this strain through to a commercial reality will require demonstration of efficacy against a broader number of pathosystems. If commercialised, however, it could contribute to an integrated management system for myrtle rust.

3.5 Recommendations from Novel anti-microbial antagonists

Bacillus sp. strain 776 has shown promise equivalent to, or greater than Bacstar, in its ability to control myrtle rust. These recommendations are next steps along a commercial pipeline.

- Further testing of Bacillus sp. strain 776 against a greater number of pathosystems to determine its ability as a broad-spectrum biocontrol agent
- Assessment of bulk production traits, tolerance to abiotic stress (UV, desiccation) and compatibility with conventional synthetic fungicides

3.6 References

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4 Outdoor potted plant experiments (Pukekohe)

4.1 Introduction

Synthetic fungicides, which are used in conventional crop protection, have been shown to be very effective for managing myrtle rust, both overseas (Martins et al. 2011) and in New Zealand (Adusei-Fosu et al. 2019, Pathan et al, 2020, Beresford & Wright 2022). However, some stakeholders, including some Māori groups, land managers and home gardeners, are philosophically opposed to using synthetic fungicides because of concerns about negative impacts on human, animal and environmental health and the possibility of fungicide resistance. Some alternative products, such as sodium bicarbonate (baking soda) are being used but there is only anecdotal evidence that they are effective and they have not been scientifically tested.

To make robust recommendations about the efficacy and suitability of potentially useful alternative products, outdoor trials under natural conditions are necessary. Testing must also compare alternative products with conventional synthetic fungicides to give a perspective on whether any efficacy they have is sufficient to be useful. In addition, many chemicals can cause plant damage (phytotoxicity) and this must also be part of any investigation into alternative products.

The non-synthetic fungicides tested as alternative products in the outdoor trials described here include the following:

- ACVM registered materials based on inorganic compounds (copper- and sulphur-based fungicides, lime sulphur, potassium silicate, potassium fatty acid soap and potassium bicarbonate). Some of these are already used in conventional crop protection.
- ACVM registered biologicals (the *Bacillus subtilis* based products, Serenade Optimum and Bacstar), used to assist suppression of some plant diseases in conventional crop protection.
- Non-registered products, including sodium bicarbonate, plant oil extracts (rosemary oil and aniseed oil), and chitosan-derived polysaccharide bioactive (PB) fractions. For this study, approval to use these materials was obtained from the Environmental Protection Authority (EPA).

Selection of products to use in the outdoor trials was determined by the combined need to gain further information on application rates for previously tested products and to respond to suggestions about further products to test.

4.1.1 Previous outdoor testing of alternative products in New Zealand

During 2022–23, PFR funded two replicated outdoor trials on chemical control of myrtle rust at its Pukekohe Research Centre in potted *Lophomyrtus* sp. ‘Red Dragon’ plants exposed to natural infection. These evaluated the efficacy of selected alternative products and synthetic fungicide groups not previously tested on New Zealand native species. Full results of these trials are presented in Appendix 1 but the main findings are summarised here because they provided important background for the planning of the MPI-funded trials in the current project.

Trial 1 2022–23. Alternative products tested were sodium bicarbonate (food grade) at two application rates (1% and 5%) and label rates of the ACVM registered products, potassium bicarbonate (0.5%),

potassium fatty acid soap (1%), potassium silicate (0.5%) and copper oxide (0.35%). The synthetic fungicide triadimenol (Group 3 DMI (demethylation inhibitor)) was used as a positive control and water as a negative control. Other synthetic fungicides were also included in the trial.

Copper oxide at the label rate had high efficacy against shoot infection and caused negligible phytotoxicity. Sodium bicarbonate had moderate efficacy at the 1% and 5% rates but the 5% rate caused severe phytotoxicity. Potassium bicarbonate, potassium fatty acid soap and potassium silicate all had slight efficacy without significant phytotoxicity.

Trial 2 2023. We further tested sodium bicarbonate at two application rates (1% and 1.5%) and potassium bicarbonate at three rates (0.5%, 1% and 1.5%) to find the rate that optimised the trade-off between rust control and phytotoxicity. Sodium bicarbonate at both rates gave slight suppression of shoot infection and good suppression of shoot dieback, however there was significant phytotoxicity at 1.5%, confirming that 1% is the highest rate that this product should be used at. Potassium bicarbonate at 0.5% gave slight suppression of shoot infection and good suppression of dieback but at the higher rates (1% and 1.5%) caused significant phytotoxicity. This confirmed that 0.5% is the highest rate at which potassium bicarbonate should be used.

4.1.2 Aims for the 2023–25 outdoor trials

The aims for the outdoor trials in the current project were to use potted plant experiments to evaluate possible alternative products chosen according to either existing ACVM registration, previous outdoor trial performance or recommendations on novel products from glasshouse testing at PFR, Ruakura. Both disease control efficacy and phytotoxicity were evaluated under natural myrtle rust infection using the susceptible Myrtaceae host *Lophomyrtus* sp. ‘Red Dragon’. From the findings, user-friendly guidelines on appropriate use of effective alternative products are to be developed.

The outdoor experiments in both 2023–25 and 2022–23 aimed to test products in a protective context, where repeated spray applications were begun prior to rust establishment in the trial plots. In the case of already established rust infection, alternative products, which generally only have protective and not curative activity, would perform less well, as was seen in the 2021–22 risk-based management study by PFR (Beresford & Wright 2022).

4.2 Materials and methods

Three outdoor trials were conducted at PFR, Pukekohe using potted plants of *Lophomyrtus* sp. ‘Red Dragon’ which were clonally propagated from cuttings at PFR, Ruakura and re-potted into 30-cm pots prior at the commencement of each trial (Figure 11).

Figure 11. Potted *Lophomyrtus* sp. 'Red Dragon' plants at The New Zealand Institute for Plant and Food Research Limited, Pukekohe in Trial 2 2024.

Each trial was a randomised complete block design with nine treatments and nine replicate blocks (Figure 12). Each treatment plot used a single potted plant with 90–120 actively growing shoots at the start of each trial. Myrtle rust developed in each trial from natural infection by airborne *A. psidii* spores. At the beginning of Trial 1, artificial inoculation of the plants was attempted by spraying a water suspension of *A. psidii* urediniospores (2 x 10^5 per mL) to try to minimise the spatial variability in rust that had occurred in earlier trials.

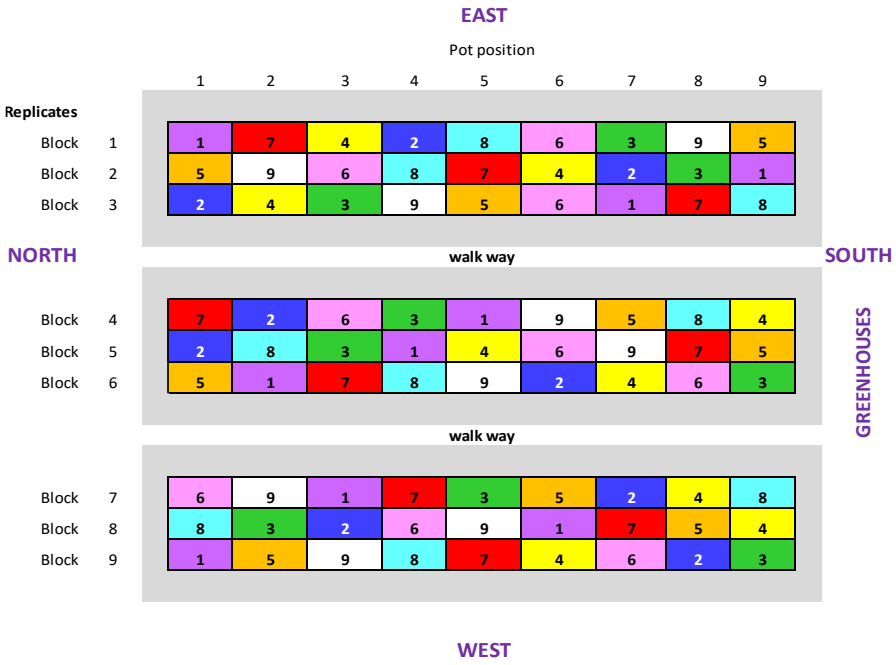

Figure 12. Example trial plan for the Pukekohe myrtle rust trials, showing the randomised layout of treatments (1–9) and replicate blocks (1–9) for Trial 2 (February to April 2024). Trials 1 and 3 used a similar arrangement.

All treatment sprays were applied by hand-held pressure sprayer at 344.7 kPa with spray drift between plants avoided by grouping treatments away from the trial during spraying then returning the pots to their randomised positions. The non-ionic surfactant, Actiwett® at 0.25 mL/L of water, was added to all treatment sprays (including the Water Control), but not to the plant oil extracts.

4.2.1 Use of experimental control treatments

The phenomenon of inter-plot interference (Paysour & Fry 1983) in replicated, randomised field trials can affect conclusions about the efficacy of treatments with marginal efficacy against a plant disease. Presence of untreated plants (negative controls) increases inoculum load locally in the trial, increasing disease pressure on adjacent plants, potentially causing underestimation of efficacy, particularly if the adjacent plants receive a low-efficacy treatment. Conversely, presence of plants treated with a highly effective fungicide (positive control) can decrease inoculum load on adjacent weaker treatments leading to overestimation of their efficacy. Inter-plot interference can be reduced by excluding positive and/or negative controls. The downside of excluding a negative control is loss of information about the potential disease that could develop in the trial and the downside of excluding a positive control is loss of information about the efficacy of the tested products compared with disease control by products with known high efficacy. Inter-plot interference is not a concern in glasshouse experiments using controlled inoculation because treatment outcomes are not affected by repeated infection cycles of the pathogen.

In the present trials, Trial 1 included only a positive control (protective and curative Group 3 DMI fungicide triadimenol), Trial 2 excluded both positive and negative controls, and Trial 3 included both a positive control (triadimenol) and a negative control (water + surfactant).

4.2.2 Trial assessments

Myrtle rust was monitored in each trial every 2 weeks after trial setup and a data collection assessment was made at a time in each trial when substantial myrtle rust symptoms had developed and shoot tip dieback was occurring. Spray and assessment dates are shown in Table 13.

Table 13. Spray dates and trial durations for three myrtle rust outdoor trials conducted at PFR Pukekohe between December 2023 and January 2025.

Trial	Spray #	Spray date
1. 2023–24	1	14-Dec-23
	2	28-Dec-23
	3	11-Jan-24
	4	25-Jan-24
	5	8-Feb-24
Data collection	14-Feb-24	
Trial duration (days)	62	
2. 2024	1	22-Feb-24
	2	7-Mar-24
	3	21-Mar-24
	4	4-Apr-24
Data collection	17-Apr-24	
Trial duration (days)	55	

Trial	Spray #	Spray date
3. 2024–25	1	29-Oct-24
	2	12-Nov-24
	3	26-Nov-24
	4	10-Dec-24
	5	24-Dec-24
	6	7-Jan-25
Data collection	15-Jan-25	
Trial duration (days)	78	

Interpreting treatment effects for myrtle rust in outdoor experiments is complex because plant growth, increasing disease intensity over time and treatment applications all interact to influence visible shoot infection, shoot tip dieback and further plant growth. The following variables were collected in each trial to allow interpretation of the temporal effects on disease and potential plant damage (phytotoxicity) caused by the products applied.

1. **Percentage of shoots in each treatment infected by myrtle rust** (lower percentage score indicates higher efficacy). Myrtle rust infection was identified by presence of spore pustules, initially yellow and later turning white or grey.
2. **Percentage of shoots with shoot tip dieback** (lower score indicates higher efficacy). Dieback developed late in the trials as spore load increased during repeated infection cycles.
3. **Percentage of shoots with new growth flush**. When used in conjunction with shoot infection and shoot dieback, new flush data help identify treatments that cause phytotoxicity, as follows:
    a. A product with high efficacy and no phytotoxicity shows little or no shoot infection, no dieback and strong growth flush.
    b. A product with high efficacy and high phytotoxicity tends to show low shoot infection and low or high dieback, depending on how phytotoxicity manifests, and no growth flush.
    c. Products with low efficacy and either low or high phytotoxicity would produce high shoot infection, high dieback and low growth flush because of rust and/or phytotoxic damage to the growing shoots. Such products would be of no interest for myrtle rust control.

4.2.3 Treatments

The treatments used in the three 2023–25 trials are shown in Table 14. Certain products were repeated in more than one trial to provide a relative measure of product performance between trials. Some of the alternative products were tested at different application rates to explore trade-offs between efficacy and phytotoxicity.

Common salt (sodium chloride) was included in 2024 Trial 2 to test the hypothesis that sea salt spray inhibits myrtle rust infection in coastal areas.

The treatment identifiers used are shown in Table 14 and Figures 13-15 include application rates only for treatments where more than one rate was used over all the trials, including the 2022–23 trials. For a complete list of products, registrants/suppliers, active ingredients and application rates, see Appendix 2 (2022–23 trials) and Appendix 3 (2023–25 trials).

Table 14. Spray treatments used in three myrtle rust outdoor trials conducted at PFR Pukekohe between December 2023 and January 2025.

Trial #	Treatment #	Treatment identifier	Product	Active ingredient	Product appl. rate (mL or g/L)	Product appl. rate (%)
Trial 1	1	Triadim	Vandia® EC	Triadimenol	1	0.1
2023-24	2	Serenade	Serenade® Optimum	*Bacillus subtilis*	2.5	0.25
	3	Bacstar® 2 g	Bacstar®	*Bacillus subtilis*	2	0.2
	4	CuOCl	Yates Copper oxychloride	Copper oxychloride	2.5	0.25
	5	CuO	Nordox™	Copper oxide	1.7	0.17
	6	CuOH	Kocide® Opti™	Copper hydroxide	3.12	0.312
	7	Sulph 0.3%	Kiwicare® Organic Super Sulphur	Sulphur	3	0.3
	8	Lime sulf 0.5%	Yates Lime sulfur	Calcium polysulfide	5	0.5
	9	Pot bicarb 0.5%	OCP Eco-fungicide™	Potassium bicarbonate	5	0.5
Trial 2	1	Rosemary oil 1%	Rosemary oil 1%	Rosemary oil	10	1
2024	2	Aniseed oil 1%	Aniseed oil 1%	Aniseed oil	10	1
	3	Salt 1%	Sea salt 1%	Sodium chloride	10	1
	4	Bacstar 2g	Bacstar	Bacillus subtilis	2	0.2
	5	Bacstar 4g	Bacstar	Bacillus subtilis	4	0.4
	6	Lime sulf 0.5%	Yates Lime sulfur	Calcium polysulfide	5	0.5
	7	Lime sulf 1%	Yates Lime sulfur	Calcium polysulfide	10	1
	8	Sulph 0.3%	Kiwicare® Organic Super Sulphur	Sulphur	3	0.3
	9	Sulph 0.6%	Kiwicare® Organic Super Sulphur	Sulphur	6	0.6
Trial 3	1	Chit PB-2 1%	Chitosan PB-2	Chitosan	10	1
2024-25	2	Chit PB-2 2%	Chitosan PB-2	Chitosan	20	2
	3	Chit PB-4 1%	Chitosan PB-4	Chitosan	10	1
	4	Chit PB-4 2%	Chitosan PB-4	Chitosan	20	2
	5	Pot bicarb 0.5%	OCP Eco-fungicide™	Potassium bicarbonate	5	0.5
	6	Sod bicarb 1%	Baking soda (1%)	Sodium bicarbonate	10	1
	7	Sulph 0.3%	Kiwicare® Organic Super Sulphur	Sulphur	3	0.3
	8	Triadim	Vandia® EC	Triadimenol	1	0.1
	9	Water Con	Water	Water + surfactant	–	–

4.2.4 Statistical analyses

In all trials, ANOVA was performed on each of the assessed variables using Genstat (Release 24.1 11 February 2025) with mean separation by the Bonferroni method ($\alpha = 0.05$). Analyses were performed on either the percentage scores or logit transformed percentage scores in cases were the ANOVA assumptions of normally distributed residuals and homogeneous variance were poorly met. Logit transformation was only required in Trial 3 2024 for % shoots with dieback (Figure 3.5), where the bars in the graph are back-untransformed logit means.

4.3 Results

There was considerable spatial variability in rust intensity in all trials because foci infection developed in certain parts of each trial. This caused high variability between the replicate blocks and limited the ability to identify statistically significant treatment effects. In anticipation of this problem, Trial 1 had been inoculated with *A. psidii* at the beginning of the trial under what appeared to be ideal overnight conditions for infection. However, the appearance of rust symptoms in patches across the trial suggested the inoculation did not help to reduce rust variability. No further trial inoculations were attempted.

4.3.1 Trial 1 2023–24

With absence of a water control, Trial 1 had low disease pressure and consequently little dieback developed, except for the 0.5% potassium bicarbonate treatment, where phytotoxicity induced severe dieback and growth flush suppression (Figure 3.3).

The three copper formulations, copper oxychloride (CuOCl), copper oxide (CuO) and copper hydroxide (CuOH) gave good suppression of shoot infection and dieback, like the positive control triadimenol. However, there was no significant difference in infection suppression between them but there was a trend for growth flush to rank CuOH > CuO > COCl, suggesting that CuOH was the least phytotoxic of the copper products at the label application rates used.

Sulphur at 0.3% (label rate) was effective in suppressing shoot infection and dieback, although it did appear a have a slight, although non-significant, tendency to suppress growth.

Lime sulphur at 0.5% gave significantly less suppression of shoot infection than triadimenol and the copper compounds but little dieback developed. This may have been the result of low disease pressure in the plots rather than high efficacy of lime sulphur.

Potassium bicarbonate at 0.5% (recommended rate) was associated with high shoot infection, suggesting poor efficacy, and very high dieback and growth flush suppression, suggesting phytotoxicity. This was similarly detected in 2022–23 Trial 1 (Appendix 1) for the 0.5% rate and there was even greater phytotoxicity in 2023 Trial 2 (Appendix 1) at 1% and 1.5%. In that trial the 0.5% rate did not cause significant phytotoxicity.

The biologicals Serenade and Bacstar gave slight suppression of shoot infection, with Serenade significantly less effective than Bacstar. For dieback suppression, they were not significantly different, although Serenade tended to be associated with greater dieback than Bacstar. Serenade also suppressed growth flush significantly more than Bacstar and was not significantly different from 0.5% potassium bicarbonate in that regard. It appears, therefore, that Bacstar has slight efficacy against myrtle rust and Serenade is less efficacious than Bacstar and tends to cause greater phytotoxicity.

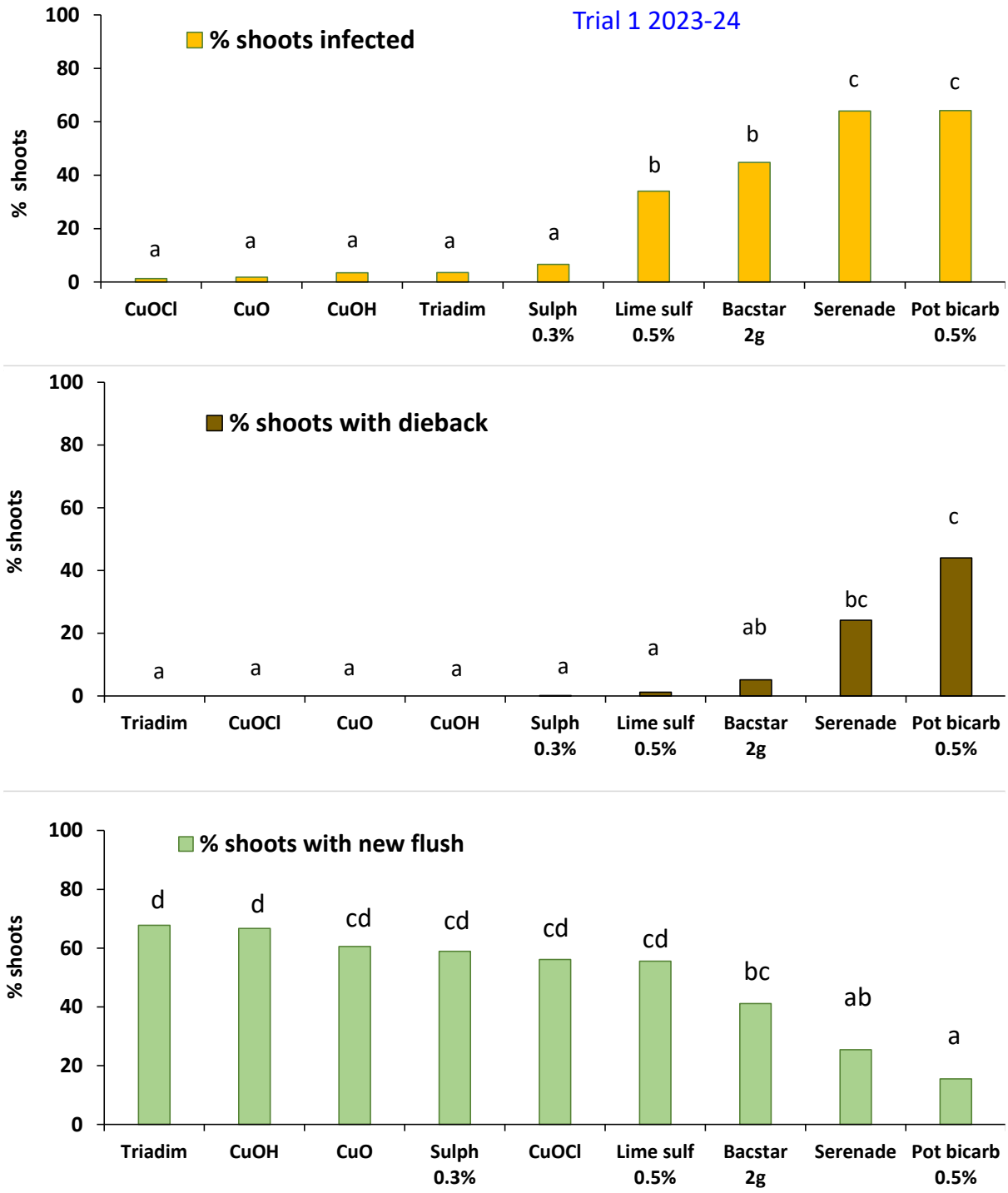

Figure 13. Trial 1 2023–24 results collected on 14 February 2024 showing Top: percentage of shoots infected with myrtle rust; Middle: percentage of shoots with tip dieback; Bottom: percentage of shoots with new flush growth. Analysis of variance (ANOVA) F-probability for all analyses $p < 0.001$. Within each graph, bars accompanied by the same letter are not significantly different (Bonferroni test, $\alpha = 0.05$).

4.3.2 Trial 2 2024

Trial 2 did not include negative or positive controls and this led to few significant treatment differences compared with trials where one or both these controls were used. However, omission of these controls made this trial more likely to reveal true differences between the treatments.

Salt gave very poor suppression of shoot infection, was associated with severe dieback and severely inhibited growth flush (Figure 3.4). Salt therefore appears to have a negligible fungicidal effect on myrtle rust and is highly phytotoxic.

Rosemary oil and aniseed oil gave slight suppression of shoot infection and dieback, which was not significantly different from most of the other treatments. This suggests these plant oils do have slight efficacy against myrtle rust and there appeared to be a slight tendency for them to suppress growth flush.

Bacstar performed similarly in terms of shoot infection, dieback and growth flush at both the recommended label rate of 2 g/L and the double rate of 4 g/L. This suggests the label rate provides the maximum potential performance for this product against myrtle rust.

The comparison of sulphur at label rate (0.3%) and double rate (0.6%) suggested slightly stronger control of both shoot infection and dieback at 0.6%, although the difference was not significant. There was similarly a non-significant trend for more growth suppression by sulphur at the higher rate.

For lime sulphur at the label rate (0.5%) and double rate (1%), there was slightly greater (not significant) control of shoot infection at the higher rate but growth flush suppression was greater at the lower rate, which was surprising. Together with the Trial 1 2023–24 results it appears lime sulphur has weak efficacy against myrtle rust and is slightly phytotoxic.

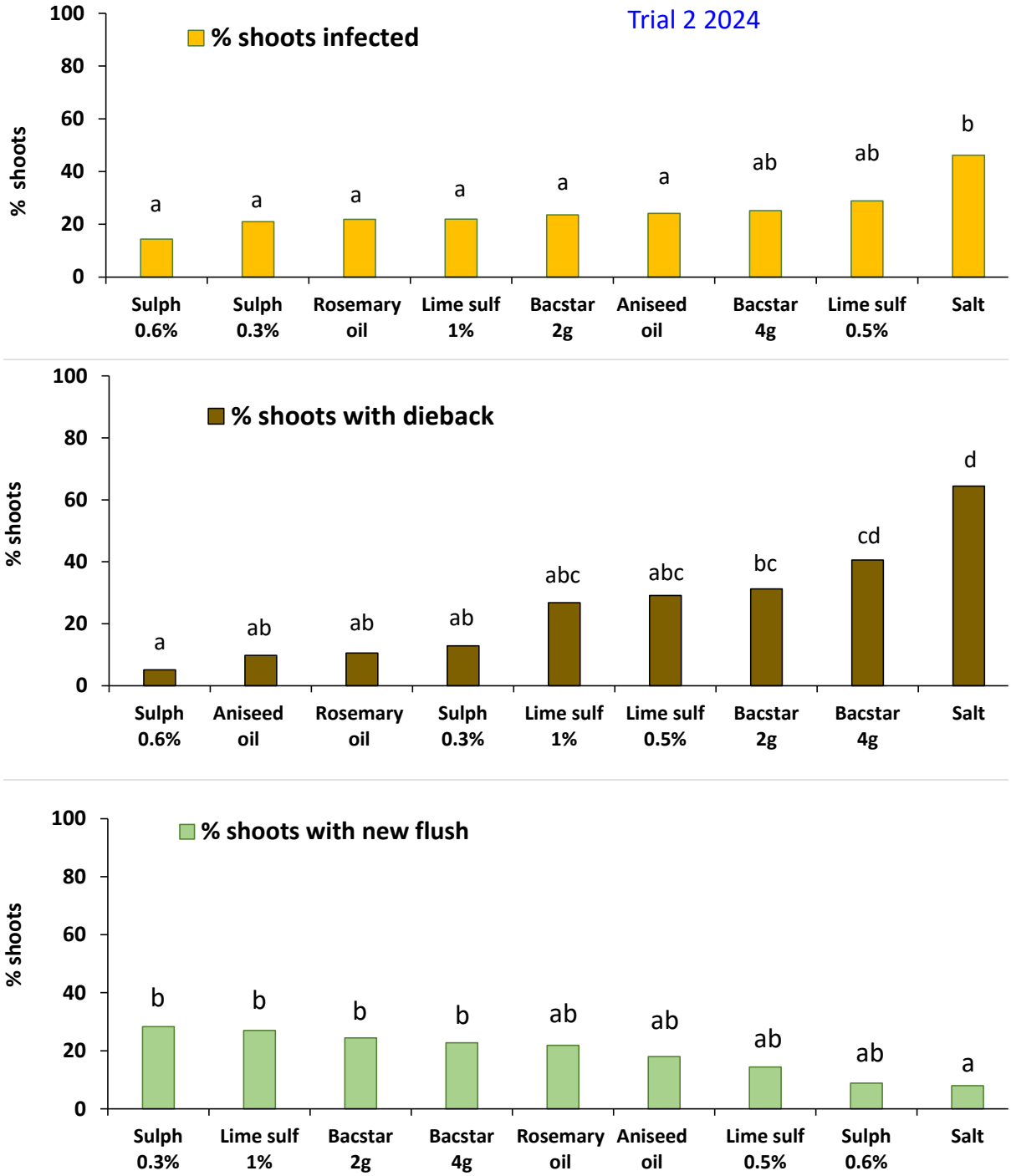

Figure 14. Trial 2 2024 results collected on 17 April 2024 showing Top: percentage of shoots infected with myrtle rust; Middle: percentage of shoots with tip dieback; Bottom: percentage of shoots with new flush growth. Analysis of variance (ANOVA) F-probability for % shoots infected = 0.002, for other analyses $p < 0.001$. Within each graph, bars accompanied by the same letter are not significantly different (Bonferroni test, $\alpha = 0.05$).

4.3.3 Trial 3 2024–25

Trial 3 focussed on comparing two different chitosan bioactive fractions from the Ruakura glasshouse assays at application rates of 1% and 2% with the previously tested products sodium bicarbonate, potassium bicarbonate and sulphur at standard rates. Both negative and positive controls (water and triadimenol) were included.

The water control had severe shoot infection that was significantly greater than the other treatments, showing the high potential for myrtle rust development in that trial (Figure 15). The severe disease also severely suppressed growth flush.

There appeared to be a rate effect for control of shoot infection and dieback by both chitosan PB-2 and PB-4, with the 2% rate ranking better than the 1% rate, although the differences were not significant. There was no suggestion of greater phytotoxicity at the higher rate, in fact the converse appeared to be true, although the low growth flush in the 1% treatment may have been caused by the higher shoot infection. There was no evidence that one fraction performed better than the other for either disease control or phytotoxicity.

Sodium bicarbonate at 1% and potassium bicarbonate at 0.5% (label rate) showed slight suppression of shoot infection and they were not significantly different from each other or from the chitosan products.

Sulphur at 0.3% gave significantly greater suppression of shoot infection than 1% sodium bicarbonate and, for dieback, it was not significantly different from triadimenol.

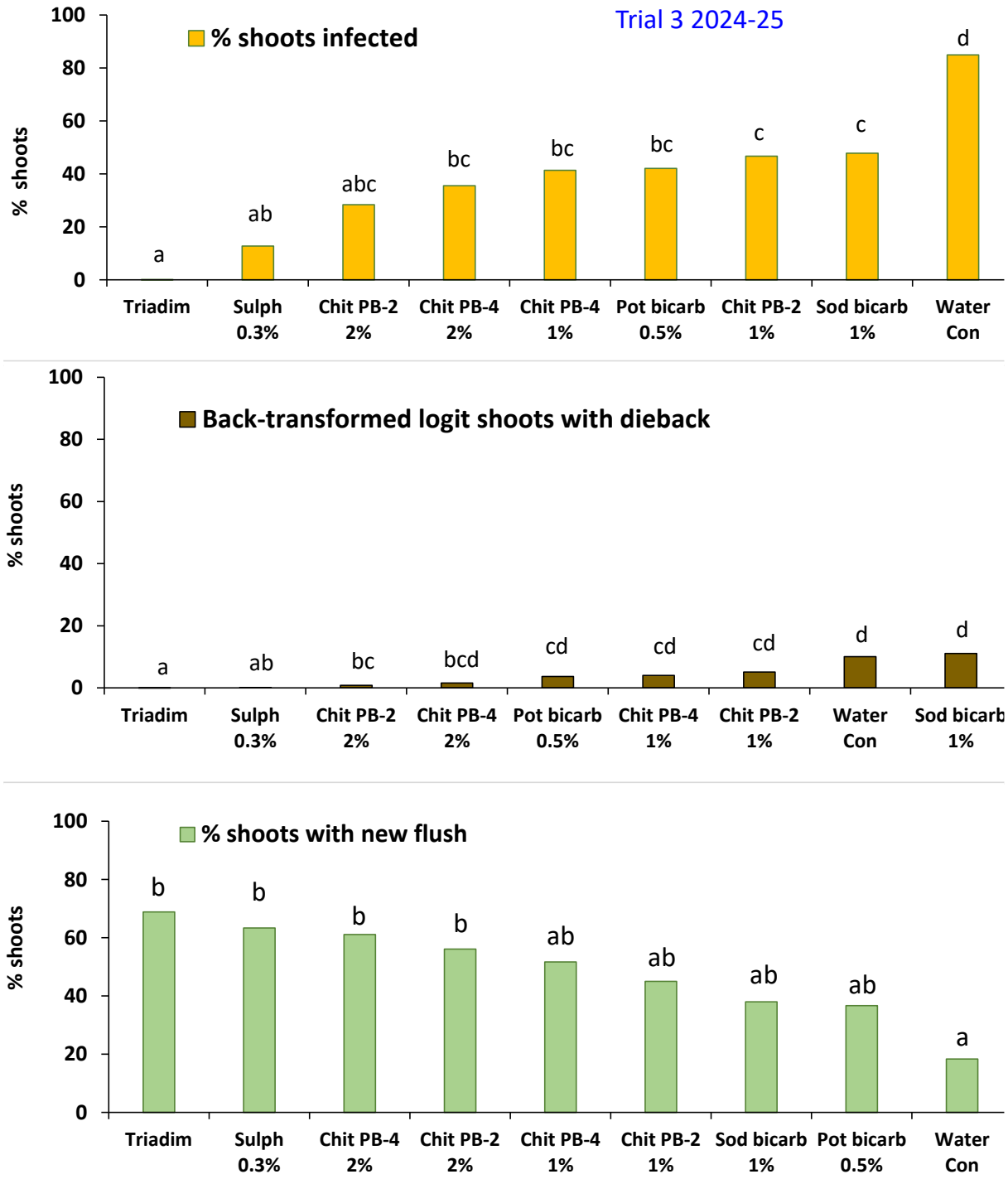

Figure 15. Trial 3 2024–25 results collected on 15 January 2025 showing Top: percentage of shoots infected with myrtle rust; Middle: percentage of shoots with tip dieback back-transformed from logit means; Bottom: percentage of shoots with new flush growth. Analysis of variance (ANOVA) F-probability for all analyses $p < 0.001$. Within each graph, bars accompanied by the same letter are not significantly different (Bonferroni test, $\alpha = 0.05$).

4.4 Discussion

For this project, two outdoor experiments were originally planned, however because the first trial was started promptly in December 2023 it allowed time for a second trial in autumn 2024 and a third in spring-summer 2024-–25. The third trial towards the end of the project enabled us to field-test the chitosan products that showed promise in the glasshouse assays. The three trials evaluated a total of 21 treatments and, in addition, a further 10 treatments had been evaluated in the 2022–23 trials, giving a total of 31 treatments comprising 23 different treatments (including water), 16 of which were alternative to synthetics. Six synthetic fungicides were included to give a wider perspective on performance of the alternative products.

The trials collected data on efficacy and phytotoxicity of each product tested and, for products where phytotoxicity was a particular risk, more than one application rate was used to find a optimised rates that maximised efficacy and minimised phytotoxicity.

Performance of the tested products ranged widely. Figure 16 gives a colour coded semi-subjective summary of myrtle rust efficacy and phytotoxicity for all 32 treatments (including water) from the five 2022–2023 and 2023–2025 trials. These ratings can be divided into three performance categories as a guideline to which products may have practical utility in managing myrtle rust:

Category A Efficacy 8–10 and phytotoxicity 1–3. These products are highly effective with negligible risk of plant damage due to phytotoxicity. They include the synthetic fungicides, the three copper fungicides and sulphur at 0.3% but not 0.6%, which would risk plant damage.

Category B Efficacy 5–7 and phytotoxicity 4–6. These products have slight efficacy and some risk of phytotoxicity, depending on application rate. They include sulphur at 0.6%, lime sulphur, the chitosan products, particularly at the 2% rate, rosemary and aniseed plant oils and the *Bacillus subtilis* biological, Bacstar. These could be useful in low disease risk situations such as on more resistant hosts (e.g., mānuka seedlings) and more susceptible species during the low-risk season, e.g., late autumn to early spring (May to September).

Category C Efficacy 3–4 and/or phytotoxicity 7–8. These products have poor activity against myrtle rust and there is a risk of plant damage due to phytotoxicity, depending on application rate. Sodium bicarbonate at 1% and potassium bicarbonate at 0.5% (label rate) were in this category in some trials but were in Category D in other trials. At higher application rates they were more likely to be in Category D because of phytotoxicity.

Category D Efficacy 1–2 and/or phytotoxicity 9–10. These products have negligible activity against myrtle rust and have phytotoxicity risks at rates at which they are fungicidal. They include sodium bicarbonate at application rates above 1%, potassium bicarbonate at application rates above 0.5%, potassium soap, potassium silicate and common salt.

	Product		Myrtle rust efficacy					Phytotoxicity				
			2023-2025			2022-2023		2023-2025			2022-2023	
			Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2
1	Synthetic fungicides	*Triadimenol	10		10	10	10	1		1	1	1
2		*Fluxapyroxad				8	9				1	1
3		*Isopyrazam				10	10				1	1
4		*Carbendazim				8					1	
5		*Benzovindiflupyr					10					1
6		*Fluopyram					10					1
7	Copper fungicides	*CuO	10			10		2			1	
8		*CuOCl	10					3				
9		*CuOH	9					1				
10	Sulphur fungicides	*Sulph 0.3%	9	7	9			2	2	2		
11		*Sulph 0.6%		8					6			
12		*Lime sulf 0.5%	5	7				4	5			
13		*Lime sulf 1%		7					5			
14	Chitosan fractions	PB 23-2 1%			5					4		
15		PB 23-2 2%			7					4		
16		PB 23-4 1%			5					4		
17		PB 23-4 2%			7					4		
18	Plant oils	Rosemary 1%		6					5			
19		Aniseed 1%		6					5			
20	Biologicals	*Bacstar 2g	5	6				5	5			
21		*Bacstar 4g		5					5			
22		*Serenade	4					4				
23	Bicarbonates	Sod bicarb 1%			1	3	3			7	7	7
24		Sod bicarb 1.5%					4					9
25		Sod bicarb 5%				7					10	
26		*Pot bicarb 0.5%	2		3	1	2	9		7	8	9
27		*Pot bicarb 1%					2					8
28		*Pot bicarb 1.5%					2					10
29	Miscellaneous	*Potassium soap				2					5	
30		*Potassium silicate				1					8	
31		Salt 1%		1					10			
32		Water			1	1	1			10	8	9
		No. treatments	9	9	9	11	11	9	9	9	11	11

*Registered by the Agricultural Compounds and Veterinary Medicines (ACVM) Group, Ministry for Primary Industries (ACVM 2025)

	Performance categories									
	Category A			Category B			Category C		Category D	
Myrtle rust efficacy	10	9	8	7	6	5	4	3	2	1
Phtyotoxicity	1	2	3	4	5	6	7	8	9	10

Figure 16. Colour coded product performance categories based on a semi-subjective numerical rating (1–10) for efficacy of myrtle rust control and phytotoxicity for 31 treatments used in five outdoor trials, three from 2023–25 and two from 2022–23. Green represents best performance and red represents worst performance for both efficacy and phytotoxicity. Product application rates are included for products used at more than one rate. Other Agricultural and Veterinary Medicines Group of Ministry for Primary Industries (ACVM)-registered products were used at label application rates.

To our knowledge, this study is the first report evaluating a wide range alternative products for myrtle rust control, although use of copper-based fungicides, which in some contexts would not be considered alternative products, has previously been reported both overseas (Ferrari et al. 1997, Goes et al. 2004) and in New Zealand (Beresford & Wright 2022).

Goes et al. (2004) demonstrated in Brazil that copper oxychloride, copper hydroxide and copper oxide applied pre-infection in the field for control of myrtle rust on guava (*Psidium guajava*) were as effective as the systemic Group 3 DMI, tebuconazole. However, Ferrari et al. (1997) in Brazil reported that copper oxychloride did not significantly reduce myrtle rust on guava and Pathan et al. (2020), in New Zealand, also reported copper oxide to be ineffective. The lack of effectiveness in the latter two cases could be explained if the copper fungicides, which have only protective activity, were used against already established infection. This raises the important point that all the alternative products tested

here are assumed to have only protective activity and not curative (systemic) activity, therefore it is critical that they are applied before myrtle rust infection becomes established.

All the ACVM-registered alternative products reported in these trials can be used under off-label use conditions to control myrtle rust, but users are responsible for managing any risks, including residues, health and environmental considerations (NZGAP 2023). The non-ACVM-registered alternative products (chitosans, plant oils and sea salt) are exempt from registration if used on Myrtaceae plants that are not for human or animal food production, but the user is responsible for managing any risks. Most Myrtaceae plants growing in New Zealand are not food plants, however, for feijoa and any other Myrtaceae plant used for human or animal food production, non-ACVM-registered cannot be used.

The Category B products, which contain the two chitosans, rosemary and aniseed oils and the biological, Bacstar, showed slight efficacy, although substantially less than the Category A products, without causing phytotoxicity. Another plant oil, the ACVM-registered Timorex Gold® (tea tree oil; *Melaleuca alternifolia*) was tested in a controlled environment with artificial *A. psidii* inoculation and was found not to control myrtle rust (Adusei-Fosu 2019). There is a need for further work to explore efficacy and phytotoxicity of the Category B products over a range of application rates. Bacstar and Serenade Optimum are ACVM-registered and could be used immediately against myrtle rust off-label. In the short term, the uptake of the chitosans, rosemary oil and aniseed oil is dependent on suitable formulations for spray application becoming available.

Salt was included in Trial 2 2024 to inform the hypothesis that myrtle rust does not develop on pōhutukawa along northern coats because salt spray from the sea suppresses rust development. However, salt gave poor inhibition of shoot infection and caused severe growth inhibition, showing it is not very fungicidal and is very phytotoxic. It is likely that an apparent lack of myrtle rust on coastal pōhutukawa may occur because salt phytotoxicity limits new shoot growth and therefore limits availability of new plant tissue that is susceptible to infection. It is possible that salt burn is not generally observed on coastal pōhutukawa shoots because this species has evolved some tolerance to the worst of its phytotoxic effects.

The results of these trials apply directly to *Lophomyrtus* spp. but whether they apply to other vulnerable species, like *Metrosideros excelsa* (pōhutukawa) and *Syzygium maire* (maire tawake; swamp maire) should be further studied. It is likely that the myrtle rust efficacy data from these trials would apply more widely than the phytotoxicity data, which could be quite species-specific. Applicability of the results could also be seasonally dependent because of the limited period during a year when active growth that is susceptible to infection is available. Generally, there is less need to spray against myrtle rust during the cooler months. Phytotoxicity effects may also be decreased during periods when there is less active growth.

4.5 Recommendations

Of the alternative products tested in the outdoor experiments, the ACVM-registered and HSNO-approved (Hazardous Substances and New Organisms Act 1996) plant protection products used at recommended label rates, and following any controls under their HSNO approval, can be used for myrtle rust control under off-label use conditions (NZGAP 2023). The products, rosemary oil, aniseed oil, the two chitosan products and sodium bicarbonate, are not ACVM registered but could be used on non-food, ornamental and amenity plants because such use is ACVM exempt.

The alternative products tested that had useful efficacy against myrtle rust are assumed to have only protective activity and no curative (systemic) activity. It is therefore critical that treatment applications of these products are initiated in early summer before visible rust infection becomes established.

Sodium bicarbonate and potassium bicarbonate gave variable disease control which tended to be greater at higher application rates but there was also greater phytotoxicity at higher application rates. These products cannot be recommended for reliable myrtle rust control.

In using any of the products tested here, they should first be trialled on a small number of plants at the intended application rate to make sure there is no phytotoxicity. This is particularly important when using them on hosts other than *Lophomyrtus* spp.

It is important to note that the alternative products tested here do not necessarily have lower risk for human, animal and environmental health compared with appropriately used synthetic fungicides and such risks must be managed appropriately for each alternative product.

4.6 References

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5 Overall conclusions

This study took a coordinated approach to identify alternatives to conventional fungicides for use in New Zealand to manage myrtle rust in accordance with the MPI request for proposal guidelines. It is recognised that conventional synthetic fungicides can effectively manage myrtle rust, however, many stakeholders avoid the use of fungicides and opt for 'alternative' products despite lack of empirical evidence supporting their use.

The context of the project was initially directed towards the plant nursery industry, but the findings are also relevant to other interest groups wishing to manage myrtle rust without resorting to conventional fungicides (e.g. in home gardens and public areas where conventional fungicides may be inappropriate).

The outdoor potted plant trials allowed alternative products that could be of immediate benefit for myrtle rust management to be identified. Some of the products tested in outdoor trials came from the glasshouse assays at PFR, Ruakura to select potentially useful candidate materials. These were rosemary oil, aniseed oil and two chitosan products that have not previously been tested under New Zealand conditions.

In the outdoor trials, all products were used protectively, with spray applications beginning before disease had become established. This is important because all the outdoor products have protective rather than curative activity. All products were categorised according to their efficacy and tendency to cause plant damage (phytotoxicity).

The most efficacious products (Category A) were the synthetic fungicides, copper fungicides and sulphur, all of which gave a high degree of protection against myrtle rust. Products in Category B had slight efficacy and tended to have some risk of phytotoxicity, depending on application rate. The best of these were the two chitosans, rosemary and aniseed plant oils, and the biologicals, Bacstar and Serenade Optimum. Despite their limited efficacy these Category B products could be useful under low disease pressure situations, such as more resistant hosts (e.g. on mānuka seedlings) and on more susceptible species during periods of low myrtle rust risk. e.g. between late autumn and early spring (May to September).

Products with poor efficacy and a risk of phytotoxicity (Categories C and D), were sodium bicarbonate potassium bicarbonate, potassium soap, potassium silicate, and sea salt. Using these is likely to give unreliable myrtle rust control and risks plant damage and, therefore, their use is not recommended.

Although the chitosans appeared promising as alternative products, they along with rosemary oil, aniseed oil, sodium bicarbonate and sea salt, cannot legally be applied in the field for plant protection at the present time because they are not EPA approved and not ACVM-registered.

From the Lincoln potted plant assays, the promising bacterial strains (*Bacillus* sp.) appeared to have efficacy that was possibly better than Bacstar and it could potentially be included under Category B. These strains are not currently available as a formulated product and further research is required to determine the practicality of their use for myrtle rust control.

Similarly, further work is recommended to develop and optimise effective formulations for the two chitosan products, rosemary oil and aniseed oil.

Appendix 1. 2022–23 The New Zealand Institute for Plant & Food Research Limited (PFR) trials on alternative products

Background

In 2022–23, The New Zealand Institute for Plant and Food Research Limited (PFR) funded two field trials to compare a selection of both synthetic fungicides and alternative products (Table A1.1). Four synthetics were tested to add to existing data on the myrtle rust efficacy of different FRAC (Fungicide Resistance Action Committee) mode-of-action groups (FRAC 2024). The active ingredients were:

- Triadimenol, a Group 3 demethylation inhibitor (DMI). This fungicide has consistently been shown to be one of the most effective compounds against myrtle rust (Beresford & Wright 2022) and provides a good positive control for comparison with other products.
- Carbendazim, a Group 1 methyl benzimidazole carbamate (MBC). This group has not previously been tested against myrtle rust and has MBCs are highly systemic they could be useful as curative sprays if they show good activity.
- Fluxapyroxad, isopyrazam, fluopyram and Benzovindiflupyr; four Group 7 succinate dehydrogenase inhibitors (SDHI). Fluxapyroxad had appeared to have relatively poor performance in earlier trials (Beresford & Wright 2022) and it was important to investigate other members of this group because some are recommended in NZPPI (2025).

The following alternative products were also tested:

- Copper oxide (FRAC Group M1) was included for comparison with previous work using copper hydroxide against myrtle rust (Beresford & Wright 2022).
- Sodium bicarbonate and potassium bicarbonate, each at three application rates, potassium fatty acid soap and potassium silicate. Sodium bicarbonate is being used by some individuals but is not an Agricultural and Veterinary Medicines Group of Ministry for Primary Industries (ACVM)-registered product, whereas potassium bicarbonate is ACVM-registered, so it was important to understand whether the two products have comparable myrtle rust efficacy.

Materials and methods, 2022–23 trials

The two outdoor trials were conducted at PFR, Pukekohe between November 2022 and March 2023. Each trial had 11 treatments and nine replicates (Table A1.1) and used *Lophomyrtus* sp. ‘Red Dragon’ and the same procedures described above for the 2023-25 trials. The spray and assessment dates are shown in Table A1.2.

Treatments 2022–23 trials

Table A1.1. Spray treatments used in two myrtle rust outdoor trials conducted at The New Zealand Institute for Plant and Food Research Limited, Pukekohe between November 2022 January 2023.

	Treatment #	Treatment identifier	Product	Active ingredient (AI)	Product appl. rate (mL or g/L)	Product appl. rate (%)
Trial 1	1	Triadim	Vandia EC	Triadimenol	1	0.1
2022-23	2	Fluxapy	Imtrex® EC	Fluxapyroxad	3.2	0.32
	3	Carbend	Protek	Carbendazim SC	0.5	0.05
	4	Isopyra	Seguris Flexi EC	Isopyrazam	1.6	0.16
	5	CuO	Nordox 75 WG	Copper	3.5	0.35
	6	Sod bicarb 1%	Baking soda	Sodium bicarbonate (food grade)	10	1
	7	Sod bicarb 5%	Baking soda	Sodium bicarbonate (food grade)	50	5
	8	Pot bicarb 0.5%	OCP Eco-fungicide™	Potassium bicarbonate	5	0.5
	9	Pot. soap	NSA potassium soap	Fatty acids of potassium salts	10	1
	10	Pot silicate 0.5%	HML Silco	Potassium silicate	5	0.5
	11	Water Con	Water	Water + surfactant		0
Trial 2	1	Triadim	Vandia® EC	Triadimenol EC	1	0.1
2023	2	Fluxapy	Imtrex® EC	Fluxapyroxad EC	3.2	0.32
	3	Benzovind	Elatus® Plus	Benzovindiflupyr	0.5	0.05
	4	Isopyra	Seguris® Flexi EC	Isopyrazam EC	1.6	0.16
	5	Fluopy	Luna® Privelege	Fluopyram SC	0.3	0.03
	6	Sod bicarb 1%	Baking soda	Sodium bicarbonate (food grade)	10	1
	7	Sod bicarb 5%	Baking soda	Sodium bicarbonate (food grade)	50	5
	8	Pot bicarb 0.5%	OCP Eco-fungicide™	Potassium bicarbonate	5	0.5
	9	Pot bicarb 1%	OCP Eco-fungicide™	Potassium bicarbonate	10	1
	10	Pot bicarb 1.5%	OCP Eco-fungicide™	Potassium bicarbonate	15	1.5
	11	Water Con	Water	Water + surfactant	–	–

Spray and assessment dates 2022–23 trials

Table A1.2. Spray dates, data collection dates and trial durations for two myrtle rust outdoor trials conducted at The New Zealand Institute for Plant and Food Research Limited, Pukekohe in the 2022–23 season.

Trial #	Spray #	Spray date
4. 2022–23	1	10-Nov-22
	2	22-Nov-22
	3	9-Dec-22
	4	19-Dec-22
	5	30-Dec-22
Data collection	18-Jan-23	
Trial duration (days)	69	
5. 2023	1	28-Jan-23
	2	10-Feb-23
	3	24-Feb-23
	4	3-Mar-23
Data collection	7-Mar-23	
Trial duration (days)	38	

Results and discussion

Trial 1 2022–23

The four synthetic fungicides and copper oxide gave a high degree of suppression of shoot infection and dieback, although fluxapyroxad and carbendazim appeared to be a little less effective against shoot infection, however the difference was not statistically significant.

Sodium bicarbonate at the higher rate (5%) suppressed shoot infection to a high degree but caused severe phytotoxicity (high dieback and low growth flush). At the lower rate (1%) it showed a slight tendency to suppress shoot infection that was not significantly different from the water control. There was significantly less dieback at 1% than the water control and less suppression of growth flush, indicating less phytotoxicity of sodium bicarbonate at 1%. These results suggested sodium bicarbonate has a small degree of activity against myrtle but is prone to cause phytotoxicity if a high application rate is used.

Potassium bicarbonate at the 0.5% label rate did not give significantly better control of shoot infection than the water control but, like sodium bicarbonate at 1%, it did give better control of shoot dieback. For growth flush suppression, potassium bicarbonate was significantly better than the water control and not significantly different from sodium bicarbonate at 1%. Therefore, potassium bicarbonate, like sodium bicarbonate has a small degree of activity against myrtle and tends to cause phytotoxicity.

Potassium soap and potassium silicate both gave poor suppression of shoot infection, although potassium soap gave suppression of shoot dieback that was not significantly different from copper and the synthetic fungicides. These two potassium-based products are ACVM-registered and appear to have slight efficacy against myrtle rust, like that of sodium and potassium bicarbonate.

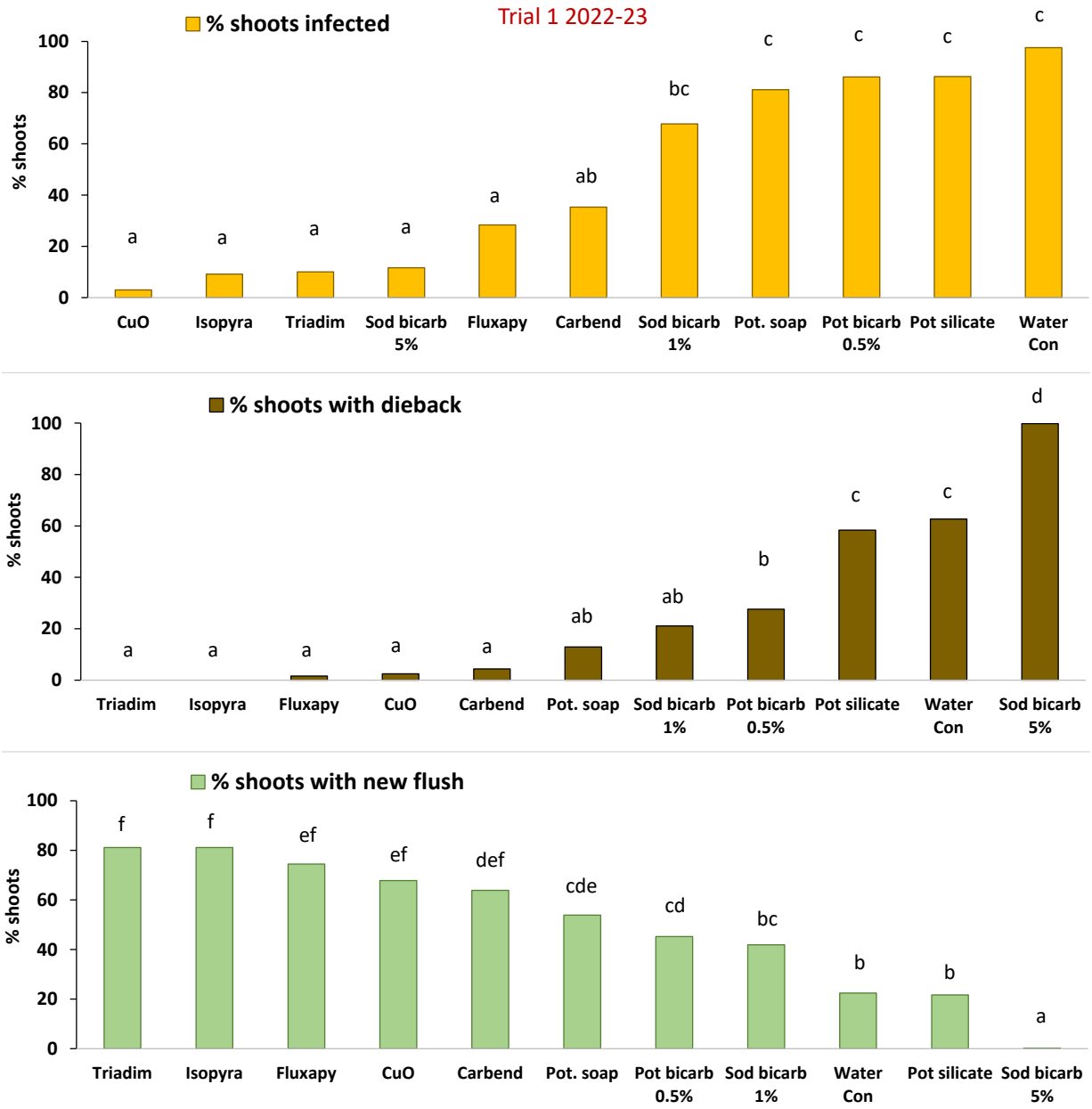

Figure A1.1. Trial 4 2022–23 results collected on 18 January 2023 showing Top: percentage of shoots infected with myrtle rust; Middle: percentage of shoots with tip dieback; Bottom: percentage of shoots with new flush growth. Analysis of variance (ANOVA) f-probability for all analyses $p < 0.001$. Within each graph, bars accompanied by the same letter are not significantly different (Bonferroni test, $\alpha = 0.05$).

Trial 2 2022–23

The four SDHIs the DMI triadimenol gave complete suppression of shoot dieback, and good suppression of shoot infection, however, the SDHIs fluxapyroxad and fluopyram did show a non-significant trend for greater shoot infection. It therefore appears that Group 7 SDHI fungicides give highly effective control of myrtle rust, although there may be slight variation in efficacy between the different active ingredients within the group.

Sodium bicarbonate at both 1% and 1.5% gave slight suppression of shoot infection and good suppression of shoot dieback, however there was significantly greater suppression of growth flush at 1.5%. This confirms that 1% is the highest rate at which sodium bicarbonate should be used to avoid phytotoxicity.

Potassium bicarbonate at the ACVM label rate of 0.5% gave slight suppression of shoot infection and good suppression of dieback but the higher rates (1% and 1.5%) gave significantly greater suppression of growth flush indicating phytotoxicity. This confirms that 0.5% is the highest rate at which potassium bicarbonate should be used.

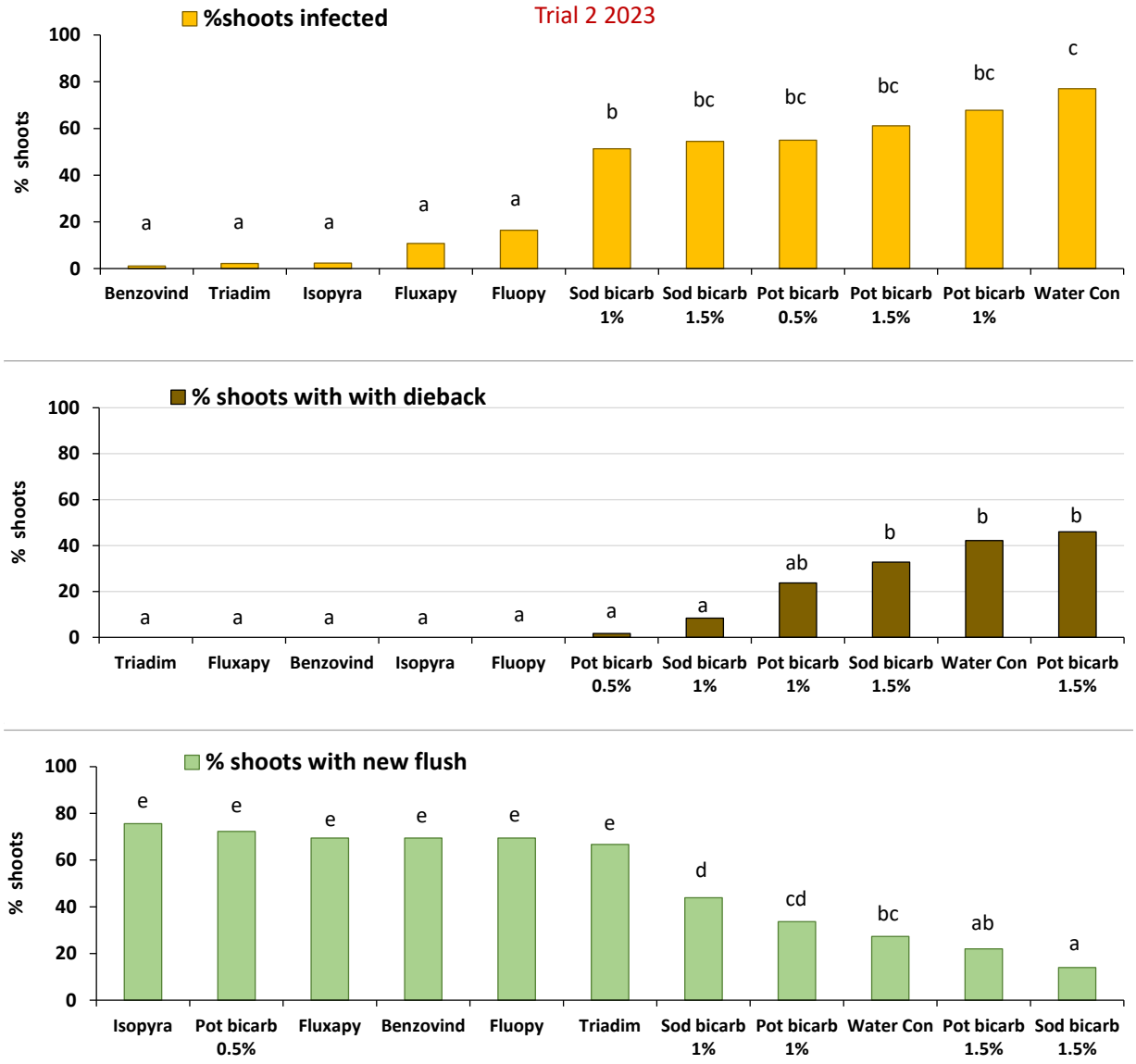

Figure A1.2. Trial 2 2023 results collected on 7 March 2023 showing Top: percentage of shoots infected with myrtle rust; Middle: percentage of shoots with tip dieback; Bottom: percentage of shoots with new flush growth. Analysis of variance (ANOVA) f-probability for all analyses $p < 0.001$. Within each graph, bars accompanied by the same letter are not significantly different (Bonferroni test, $\alpha = 0.05$).

Appendix 2. Product details for 2022–23 outdoor trials

	Treatment #	[1]FRAC MOA Group	Product	[2]ACVM Registrant / supplier	Formu-lation	Active ingredient (AI)	Prop. AI	Product appl. rate (mL or g/L)	AI appl. rate (mL or g/L)	[2]ACVM Label appl. rate (mL or g/L)
2022-23	1	3	Vandia EC	Adria New Zealand	EC	Triadimenol	0.25	1	0.25	1
Trial 1	2	BM 02	Imtrex® EC	BASF New Zealand	EC	Fluxapyroxad	0.0625	3.2	0.2	3.2
	3	BM 03	Protek	Arxada (Lonza)	SC	Carbendazim SC	0.5	0.5	0.25	0.5
	4	M1	Seguris Flexi EC	Syngenta Crop Protection	EC	Isopyrazam	0.125	1.6	0.2	1.6
	5	M1	Nordox 75 WG	Grochem/Agrinova	WDG	Copper	0.75	3.5	2.63	3.5
	6	M1	Baking soda 1%	Supermarket	SP	Sodium bicarbonate (food grade)	1	10	10	–
	7	M2	Baking soda 5%	Supermarket	SP	Sodium bicarbonate (food grade)	1	50	50	–
	8	–	OCP Eco-fungicide™ 0.5%	Dulux Group (New Zealand) Pty Ltd	SP	Potassium bicarbonate	0.94	5	4.7	1-5
	9	–	NSA (potassium soap)	Certis Belchim NV (Grochem)		Fatty acids of potassium salts	0.23	10	2.3	10-20
	10	–	HML Silco	Certis Belchim NV (Grochem)		Potassium silicate	0.44	5	2.2	2.7-5.4
	11	–	Water Control	–	–	Water+surfactant	–	–	–	–
2022-23	1	–	Vandia® EC	Adria New Zealand	EC	Triadimenol EC	0.25	1	0.25	1
Trial 2	2	BM 03	Imtrex® EC	BASF New Zealand	EC	Fluxapyroxad EC	0.0625	3.2	0.2	3.2
	3	BM 03	Elatus® Plus	Syngenta Crop Protection		Benzovindiflupyr	0.5	0.5	0.25	0.5
	4	–	Seguris® Flexi EC	Syngenta Crop Protection	EC	Isopyrazam EC	0.125	1.6	0.2	1.6
	5	–	Luna® Privelege	Bayer	SC	Fluopyram SC	0.5	0.3	0.15	0.3
	6	M2	Baking soda 1%	Supermarket	SP	Sodium bicarbonate (food grade)	1	10	10	–
	7	M2	Baking soda 1.5%	Supermarket	SP	Sodium bicarbonate (food grade)	1	15	15	–
	8	–	OCP Eco-fungicide™ 0.5%	Dulux Group (New Zealand) Pty Ltd	SP	Potassium bicarbonate	0.94	5	4.7	5
	9	–	OCP Eco-fungicide™ 1%	Dulux Group (New Zealand) Pty Ltd	SP	Potassium bicarbonate	0.94	10	9.4	10
	10	–	OCP Eco-fungicide™ 1.5%	Dulux Group (New Zealand) Pty Ltd	SP	Potassium bicarbonate	0.94	15	14.1	15
	11	–	Water Control	–	–	Water+surfactant	–	–	–	–

[1] Fungicide Resistance Action Committee; – = not classified by FRAC; MOA = mode of action.
[1] Fungicide Resistance Action Committee; – = not classified by FRAC; MOA = mode of action.
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Appendix 3. Product details for 2023–25 outdoor trials

Trial #	Trt #	[1]FRAC MOA Group	Product	[2]ACVM Registrant / supplier	[3]Formu-lation	Active ingredient (AI)	Prop. AI	Product appl. rate (mL or g/L)	AI appl. rate (mL or g/L)	ACVM Label appl. rate (mL or g/L)
Trial 1	1	3	Vandia® EC	Adria New Zealand	EC	Triadimenol	0.25	1	0.25	1
2023-24	2	BM 02	Serenade® Optimum	Bayer	WP	*Bacillus subtilis* strain QST 713	-	2.5	2.5	1-2.5
	3	BM 03	Bacstar™	UPL	WDG	*Bacillus subtilis* var. *amyloliquefaciens* strain D74	-	2	2	0.8-2
	4	M1	Yates Copper oxychloride	Dulux Group (New Zealand) Pty Ltd	WP	Copper oxychloride	0.5	2.5	1.25	3-5
	5	M1	Nordox™	AgriNova New Zealand Ltd,	WDG	Copper oxide	0.75	1.7	1.275	0.4-1.1
	6	M1	Kocide® Opti™	Corteva Agriscience	WDG	Copper hydroxide	0.3	3.12	0.936	0.7-3.12
	7	M2	Kiwicare® Organic Super Sulphur	Kiwicare Corporation Limited	WDG	Sulphur	0.8	3	2.4	1-3
	8	–	Yates Lime sulfur	Dulux Group (New Zealand) Pty Ltd	SC	Calcium polysulfide	0.2	5	1	5-13
	9	–	OCP Eco-fungicide™	Dulux Group (New Zealand) Pty Ltd	SP	Potassium bicarbonate	0.94	5	4.7	1-5
Trail 2	1	–	Rosemary oil 1%	The Essential oils of NZ Ltd	PE	Rosemary oil	1	10	10	–
2024	2	–	Aniseed oil 1%	The Essential oils of NZ Ltd	PE	Aniseed oil	1	10	10	–
	3	–	Sea salt 1%	Maldon Sea salt flakes	SP	Sodium chloride	1	10	10	–
	4	BM 03	Bacstar™	UPL	WDG	Bacillus subtilis var. amyloliquefaciens strain D747	1	2	2	0.8-2
	5	BM 03	Bacstar™	UPL	WDG	Bacillus subtilis var. amyloliquefaciens strain D747	1	4	4	0.8-2
	6	–	Yates Lime sulfur	Dulux Group (New Zealand) Pty Ltd	SC	Calcium polysulfide	0.2	5	1	5-13
	7	–	Yates Lime sulfur	Dulux Group (New Zealand) Pty Ltd	SC	Calcium polysulfide	0.2	10	2	5-13
	8	M2	Kiwicare® Organic Super Sulphur	Kiwicare Corporation Limited	WP	Sulphur	0.8	3	2.4	1-3
	9	M2	Kiwicare® Organic Super Sulphur	Kiwicare Corporation Limited	WP	Sulphur	0.8	6	4.8	1-3
Trial 3	1	–	Chitosan PBS4 23-2 1%	T Reglinsky, PFR	SP	Chitosan	1	10	10	–
2024-25	2	–	Chitosan PBS4 23-2 2%	T Reglinsky, PFR	SP	Chitosan	1	20	20	–
	3	–	Chitosan PBS2 23-4 1%	T Reglinsky, PFR	SP	Chitosan	1	10	10	–
	4		Chitosan PBS2 23-4 2%	T Reglinsky, PFR	SP	Chitosan	1	20	20	–
	5	–	OCP Eco-fungicide™	Dulux Group (New Zealand) Pty Ltd	SP	Potassium bicarbonate (0.5%)	0.94	5	4.7	1-5
	6	–	Baking soda (1%)	Supermarket	SP	Sodium bicarbonate (Food grade)	1	10	10	–
	7	M2	Kiwicare® Organic Super Sulphur	Kiwicare Corporation Limited	WP	Sulphur	0.8	3	2.4	1-3
	8	3 DMI	Vandia® EC	Adria New Zealand	EC	Triadimenol	0.25	1	0.25	1
	9	–	Water control	–	–	–	–	–	–	–

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