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Vulnerability of New Zealand Myrtaceae species to natural infection by *Austropuccinia psidii* (myrtle rust)

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Executive summary

Vulnerability of New Zealand Myrtaceae species to natural infection by *Austropuccinia psidii* (myrtle rust)

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Myrtle rust (*Austropuccinia psidii*) was first detected in Aotearoa New Zealand in 2017 and is now widely distributed in the North Island, in the north of the South Island and as far south as the West Coast and Canterbury. This invasive fungal disease infects actively growing shoots of plants in the myrtle family (Myrtaceae) and causes leaf and stem lesions, defoliation, dieback, flower and fruit death, stunted growth and, sometimes, plant death.

Current knowledge about the vulnerability of New Zealand's native myrtle species to *A. psidii* comes from resistance screening of young seedlings performed with artificial inoculation under controlled conditions, from surveillance data collected during the initial incursion response and from field observations. The controlled resistance screening is important because it can potentially reveal the inherent genetic susceptibility of species, whereas surveillance data and field observations may reflect sampling bias and other factors affecting vulnerability, such as presence of new shoot growth, presence of airborne spores (inoculum) and suitability of weather conditions. However, it needs to be determined how well the method of screening young seedlings under artificial conditions represents the effects of myrtle rust on host species in the natural environment.

We established field trials in Rotorua and Auckland in spring 2019 to compare the artificial inoculation results with myrtle rust development at two sites where inoculum and environmental conditions were relatively uniform. Each trial contained five New Zealand native Myrtaceae species: ramarama (*Lophomyrtus bullata*), rōhutu (*Lophomyrtus obcordata*) pōhutukawa (*Metrosideros excelsa*), mānuka (*Leptospermum scoparium*) and kānuka (*Kunzea robusta*) and the exotic species, rose apple (Syzygium jambos), which is highly susceptible worldwide and was included to provide an international comparison.

Monitoring of disease, plant growth and environmental conditions at the two sites over two seasons confirmed that the different species have different degrees of susceptibility and different responses to seasonal myrtle rust attack. Four species were severely affected at both sites (*L. bullata*, *L. obcordata*, *M. excelsa* and *S. jambos*), with repeated epidemics severely affecting new emerging shoots and causing severe dieback and stunted growth. The other two species (*L. scoparium* and *K. robusta*) were not affected by myrtle rust at all, except for a small incidence of fruit (seed capsule) infection observed on *L. scoparium* in Season 2. The findings about species susceptibility in the field trials broadly agreed with the artificial inoculations, except for *L. scoparium*, which had shown substantial leaf and stem susceptibility in the controlled screening, but this was not seen in the field trials.

There were other aspects of seasonal growth and species characteristics that importantly affected myrtle rust development on the highly susceptible species. These included a greater potential plant

growth rate in Auckland's warmer climate that was accompanied by more severe rust symptoms and greater damage to plants. Two separate waves of infection occurred in Auckland during summer and early autumn. There was also evidence for this in Rotorua, but not as well defined as in Auckland. In Rotorua, cooler winter temperatures allowed *A. psidii* to survive inside the host plant as latent infection throughout winter, whereas in Auckland some new symptoms could be found during warm periods during winter. We found that *M. excelsa* was slightly less susceptible than the two *Lophomyrtus* spp., with less stem infection and dieback and a slower rate of disease progression. These seasonally dynamic effects of myrtle rust damage on susceptible species would only be detectable under field conditions and they could not be identified using controlled environment screening.

Fungicides represent a way to minimise the impacts of myrtle rust. In the second season, we applied fungicides to half the plants of the four more susceptible species (*L. bullata*, *L. obcordata*, *M. excelsa* and *S. jambos*) to exclude myrtle rust and allow the impacts of the disease on plant performance to be understood. The fungicide regime started after rust was established and did not completely control infection; however, the fungicide applications still gave substantial reductions in dieback and growth stunting. The magnitude of reduction in disease impacts depended on the species and the timing of applications in relation to seasonal growth and weather conditions.

The main conclusions from this field study on myrtle rust were that:

- Susceptibility of *L. bullata*, *L. obcordata*, *M. excelsa* and *S. jambos* was high, whereas it was low in *L. scoparium* and *K. robusta* appeared to be completely resistant.
- There was evidence for a significant degree of resistance to stem infection in *M. excelsa*, although young leaves were very susceptible.
- Susceptibility screening using artificial inoculation of seedlings under controlled conditions may not accurately predict field disease outcomes in all species.
- Important aspects on the natural environment that affect vulnerability to myrtle rust include timing of seasonal growth and fruiting phenology, seasonal and regional variations in climatic conditions and species-specific changes in anatomy and physiology of plants as they age.
- Seasonal temperature appeared to be the key driver for differences in plant growth and myrtle rust development between the Rotorua and Auckland trials.
- Climate change is likely to exacerbate the impacts of myrtle rust in New Zealand in the future because rising temperatures will increase rates of *A. psidii* infection and multiplication.
- Fungicide applications were highly effective in reducing myrtle rust impacts in the Rotorua and Auckland trials. The greatest measured benefits were a reduction in shoot dieback, increased plant height growth and increased fruit survival.

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1 Introduction

Myrtle rust, caused by *Austropuccinia psidii*, is an invasive fungal disease that affects plants in the myrtle family (Myrtaceae). The pathogen was first found in Aotearoa New Zealand in early 2017 (Toome-Heller et al. 2020), and was shown to be the pandemic biotype of *A. psidii*, the same as that found in Australia and New Caledonia (du Plessis et al. 2019). Myrtle rust infection only occurs on actively growing tissues (leaves, stems, flowers and fruits), causing effects that depend on species susceptibility. These range from necrotic spots, leaf and stem lesions, defoliation and dieback to stunted growth, reduced fecundity, plant death and decline of whole plant stands (Alves et al. 2011; Carnegie et al. 2016).

Myrtle rust has now spread to many parts of the North Island, the top of the South Island, and, more recently has been found on the West Coast and in Canterbury. Of greatest concern in Aotearoa New Zealand is the impacts it will have on our native Myrtaceae, which are of traditional cultural importance to Māori and are highly valued by all. Surveillance data collected during the incursion response (Toome-Heller et al. 2020) and subsequent notifications on the iNaturalist NZ – Mātaki Taiao website (https://inaturalist.nz) identified 30 host species in New Zealand. The most frequently encountered native hosts were *Lophomyrtus bullata* (ramarama) and its hybrids with *L. obcordata* (rōhutu), and *Metrosideros excelsa* (pōhutukawa). Mānuka (*Leptospermum scoparium*) has been of great concern because of its high cultural importance and its value to the mānuka honey industry; however, it has so far not been severely affected by myrtle rust.

For a host species in the natural environment, its vulnerability to myrtle rust depends on its inherent genetic susceptibility, the presence of new host growth and environmental factors, including local airborne spore load and the suitability of weather conditions for the pathogen. Most of our current knowledge about genetic susceptibility of New Zealand native Myrtaceae is inferred from surveillance data collected during the initial incursion response and from subsequent field observations. Testing of genetic susceptibility has been done using artificial inoculation under controlled conditions in Australia on seedlings of several native species from New Zealand-collected seed. This suggested that many species are genetically susceptible, but also that there is natural resistance in some species. Whether the controlled environment information can predict the vulnerability of plants in the New Zealand environment is not currently known but needs to be determined in order for us to understand and predict the impacts of *A. psidii* on our native species.

This project aimed to compare the relative susceptibility of selected native species under natural field conditions in scientific trials at two sites where environment and spore load were relatively uniform. It also aimed to provide data on seasonal climatic factors that drive myrtle rust development to improve our understanding of how and when myrtle rust develops.

2 Materials and methods

2.1 Field trial design

Field trials were established in Rotorua, at Scion (−38.158983°, 176.263342°) in October 2019 and in Auckland at Plant & Food Research (−36.891221°, 174.727688°) in November 2019. Preparation of the sites prior to planting included weed control with glyphosate and laying down of weed mats. The seedlings were planted with 15 g of slow-release fertiliser (NPK) to facilitate their establishment. An automated drip irrigation system was installed at both sites and the plants were watered as required during summer. Each trial comprised 8 blocks of 48 seedlings (n=384 seedlings per site). A randomised block design was used with each plant family represented by at least one single tree in each block. Within blocks, plants were spaced at least 0.70 m apart, and each block was spaced 1 m from the next (Figure 1).

The trials were monitored during two myrtle rust seasons after planting. Each myrtle rust season was considered to start on 1 July and end on 30 June the following year. The first season in Rotorua comprised 9 months (October 2019 to June 2020) and in Auckland, 8 months (November 2019 to June 2020). The second season in Rotorua comprised 13 months (July 2020 to June 2021, plus July 2021) and in Auckland, 12 months (July 2020 to June 2021).

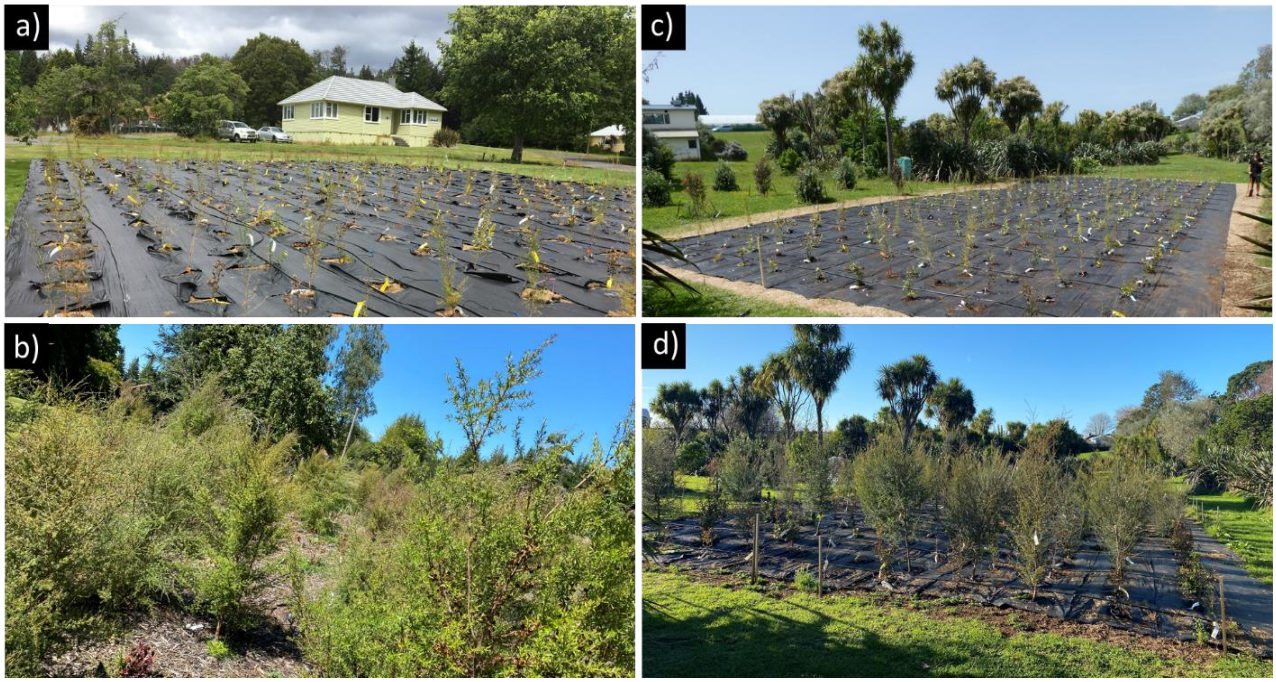

Figure 1. Myrtaceae trials during establishment and after 1 year in Rotorua (a,b) and Auckland (c,d) sites.

2.2 Study species

We selected five native species and one exotic species for the field trials. The mother plants from which seed families were raised originated from areas around Rotorua Lakes and Auckland (Table 1; Appendix 8.1). Of the five native species, *L. scoparium* and *K. robusta* had not been reported to be severely affected by myrtle rust in the field, whereas severe effects had been reported on *L. bullata* and *L. obcordata* and on young *M. excelsa* plants. *Syzygium jambos* is an exotic species that was included to provide a comparison with a species known worldwide to be highly susceptible to myrtle rust.

Table 1. Myrtaceae species, their provenance (Rotorua or Auckland) and numbers of mother plants of each included in the field trial to investigate vulnerability to myrtle rust. Eight seedlings of each mother plant were included in each of the Rotorua and Auckland plantings.

Species	Common name	Native or Exotic	No. mother plants from Rotorua	No. mother plants from Auckland	Total no. of mother plants
Leptospermum scoparium	Mānuka	Native	4	4	8
Kunzea robusta	Kānuka	Native	0	5	5
Metrosideros excelsa	Pōhutukawa	Native	0	6	6
Lophomyrtus bullata	Ramarama	Native	6	0	6
Lophomyrtus obcordata	Rōhutu	Native	9	7	16
Syzygium jambos	Rose apple	Exotic	0	1	1

Seeds from naturally pollinated plants of each species were collected from the Rotorua Lakes and Auckland areas during a previous myrtle rust study with landowner and mana whenua permissions (Smith et al. 2020; Soewarto et al. 2021).

The location of the mother trees will remain confidential as agreed with mana whenua. Half of the seeds were sent overseas to be germinated and screened against different strains of *Austropuccinia psidii* using controlled inoculation (Appendix 8.1). With mana whenua permission, the remaining progeny seeds were germinated and used for this experiment. Details of numbers of mother trees included in the Rotorua and Auckland trials are provided in Appendix 8.1.

Seedlings of *L. scoparium*, *M. excelsa*, *K. robusta* and, *L. obcordata* were 2 years old at the start of the experiment. *Lophomyrtus bullata* seedlings were 1 year old and originally derived from clones of a cultivar. Seedlings of *S. jambos* were 6 months old when planted and were sourced from seeds collected from one mother tree in suburban Auckland.

2.3 Myrtle rust disease assessment

Disease assessments started when myrtle rust first appeared in each trial, from November 2019 in Rotorua, and from January 2020 in Auckland. The assessments were conducted approximately monthly until July 2021 in Rotorua and June 2021 in Auckland, except during the COVID-19 lockdown periods.

Myrtle rust intensity was estimated visually on each individual plant on leaves, stems and shoot tips on new flush shoots with rust symptoms (either necrotic lesions, pustules, or dieback). For each monitored plant, the following disease variables were recorded:

1. **Leaf severity:** Percentage of leaf area on new shoots affected by rust in the current growth period
2. **Stem disease:** Percentage of new shoots with stem lesions in the current growth period
3. **New dieback:** Percentage of new shoots with tip dieback in the current growth period
4. **Old dieback:** Percentage of all shoots on the plant with tip dieback from previous growth periods
5. **Diseased flower/fruit:** Percentage of reproductive organs (flower buds, flowers, immature fruits, mature fruits) on the plant visibly affected by myrtle rust.

Disease incidence was derived from the disease intensity data as the percentage of plants within a factor grouping of interest with any myrtle rust symptoms. Total dieback was derived by summing new and old dieback values for each plant.

A 5-point scoring system was used to help quick decision making on percentages in the field. The severity scores were converted to percentage area affected prior to data analysis using the mid-point percentage value for each score category (Figure 2).

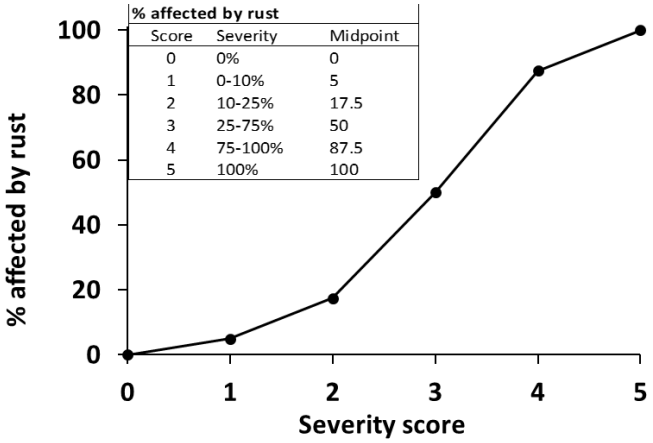

Figure 2. Myrtle rust disease score categories used for field assessment of percentages of leaf area, stems and flowers/fruit affected by myrtle rust. Percentage values were derived from the score category midpoints and were used for analyses.

2.4 Phenology assessment

Leaf and stem tissues of susceptible Myrtaceae species are only able to be infected by *A. psidii* while they are emerging and actively expanding (Glen et al. 2007; Beresford et al. 2020). Once fully expanded, these tissues become resistant to infection, which means that field epidemics of myrtle rust are limited to periods when growth flush occurs. In addition, other plant organs, such as inflorescences and fruit, which develop at certain phenological stages, may also be susceptible to infection for a period, depending on the host species. We therefore made visual estimates on each assessed plant of the following variables:

1. **New flush leaves:** Percentage of shoots with new flush leaves
2. **New flush stems:** Percentage of shoots with new flush stems
3. **Reproductive organs:** Percentage of shoots with reproductive organs at each of the four stages, flower buds, open flowers, developing fruits and mature fruits.

The same 5-point scoring system described above (Figure 2) was used for estimating percentages in the field. These assessments were undertaken monthly at the same time as disease assessments using the recording guide in Appendix 8.2.

2.5 Growth measurement

Plant height (cm) was determined monthly by measuring the height of the main stem, from the ground to the stem node of the highest fully expanded leaf.

2.6 Disease exclusion using fungicides

Disease-exclusion using fungicide applications was carried out at the Rotorua and Auckland sites during the second season of monitoring to quantify the effect of damage from *A. psidii* on New Zealand Myrtaceae. Three fungicides were selected from ones recommended by the Ministry for Primary Industries and New Zealand Plant Producers Inc. during the initial myrtle rust incursion in 2017, aided by existing knowledge of effective fungicides for control of rust fungi and availability in New Zealand (Chng et al. 2019). The three fungicides used (Table 2) belong to the demethylation inhibitor (Group 3), quinone outside inhibitor (Group 11) and succinate dehydrogenase inhibitor (Group 7) mode of action (MOA) groups. We used these three MOA groups in rotation to reduce the risk of fungicide resistance developing in the myrtle rust fungus.

Fungicides were applied to the species that showed severe myrtle rust damage in the first year. (*M. excelsa, L. bullata, L. obcordata* and *S. jambos)*. Half of the seedlings from these four species were randomly selected to be sprayed with fungicides. The other half remained untreated (controls). All foliage on treated seedlings was sprayed to the point of run-off with fungicide.

Table 2. List of fungicides used for disease exclusion.

Commercial brand	Active ingredient	[1]MOA group	Composition	Recommended rate according to label
Vandia®	Triadimenol	3	250 g/L	500 mL/ha
Flint®	Trifloxystrobin	11	500 g/kg	10 g/100 L
Sercadis®	Fluxapyroxad	7	300 g/L	20 mL/100 L

[1]Mode of action (MOA) group (Fungicide Resistance Action Committee, Europe; FRAC | Home).

Fungicides were applied using a manual pressurised backpack spray unit. Fungicide application occurred between October 2020 and May 2021 in Rotorua (10 applications) and between November 2020 and April 20201 in Auckland (eight applications) (Appendix 8.3). Application timing was determined by weather suitability, the way the different species were affected by the rust and other logistical factors associated with running the trials. The species *L. bullata*, *M. excelsa*, and *S. jambos* were sprayed between October 2020 and November 2020 in Rotorua and Auckland respectively. The *L. obcordata* plants were only sprayed in February 2021 and March 2021 for

Rotorua and Auckland respectively because of a slower establishment and growth of this species in both trials.

2.7 Weather monitoring and climatic risk

Weather monitoring equipment was installed at the Rotorua and Auckland trial sites to allow associations between seasonal myrtle rust development and weather factors to be investigated in these two climatically different regions. Previous modelling of *A. psidii* infection and latency in relation to weather variables was used to help interpret seasonal rust development in these trials. Air temperature (°C), relative humidity (%), rainfall (mm) and solar radiation (W/m^2) were measured hourly by automated weather stations (Campbell CR1000 data loggers) installed near the trials. Wetness (%) was only available from the Auckland met station.

Infection risk of myrtle rust for the Auckland and Rotorua trials was predicted using the Myrtle Rust Process Model (Beresford et al. 2018) from hourly temperature, hours/day >85% relative humidity and daily solar radiation. The model outputs relative risk values between 0 and 1, divided into five risk categories: <0.2 (very low), 0.2–0.4 (low), 0.4–0.6 (moderate), 0.6–0.8 (high) and >0.8 (very high). Days on which high or very high risk occurs are known to be associated with substantial *A. psidii* infection and less infection occurs when risk is moderate or lower (Beresford unpublished data). Actual amount of infection depends on numbers of *A. psidii* spores available to infect.

2.8 Analysis

Graphical visualisation was used to observe trends and patterns during Season 1 (November 2019–June 2020) and Season 2 (July 2020–July 2021). Statistical analysis was performed using R version 3.6.1 (R Core Development Team 2019) for Season 2, when plants were treated with fungicides.

To test the effect of fungicide treatment on plant growth and myrtle rust severity, a mixed-effects model was used to analyse three response variables on three species (*L. bullata, L. obcordata. M. excelsa*) during Season 2 (2020–2021):

1. **New flush leaves:** defined as the percentage of all shoots on a plant with actively emerging (new flush) leaves
2. **Disease severity:** defined as the percentage of new leaf area affected by myrtle rust
3. **Myrtle rust dieback:** defined as the percentage of new stems with myrtle rust dieback.

The data were analysed separately by site and fungicide application date because of differences in the frequency of fungicide application in each site and species (cf. Section 2.6). The exotic *S. jambos* was excluded from the analysis because there were insufficient samples to estimate coefficients robustly. The species *K. robusta* and *L. scoparium* were excluded from this analysis because leaves and stems were not affected by myrtle rust during the study. Plants that failed to establish (no new growth produced) or that died from reasons other than *A. psidii* infection (frost, drought, or insect damage) were excluded from the analysis. *Lophomyrtus obcordata* had a particularly large proportion of plants that either established poorly or died.

The three response variables were analysed using a generalised linear mixed model (GLMM) assuming a beta distribution based on the parameterisation of Ferrari and Cribari-Neto (2004) and logit link function. Prior to analysis, each response variable on a scale of 0–100 was converted into the

open standard unit interval (0,1) to satisfy conditions of the beta distribution by using (y ×(n-1)+0.5)/n, where y is the proportion of the response of interest on a scale of 0–1 and n is the number of observations, as recommended by Smithson and Verkuilen (2006).

The beta GLMMs for the response variable included the following fixed-effects:

- 'species' (factor with two groups: *L. bullata*, *M. excelsa* for the analysis of date of fungicide applications in November 2020–July 2021 for Rotorua and Auckland, and factor with three groups: *L. bullata*, *M. excelsa*, *L. obcordata* for the analysis of date of fungicide application in March–July 2021 for Rotorua and April–July 2021 in Auckland)
- 'Fungicide' (factor with two levels: 'untreated' and 'treated')
- 'Date of assessment' (DOA) representing the month effect (factor with different time points depending on the date of fungicide application)
- and their interaction 'species::fungicide::DOA'.

Random effects included: 'seedling' nested in 'family' in 'species' in 'block'.

Height growth rate (cm per month) was calculated by taking the difference between the initial and last height measurements (between November 2020 and June 2021 for *L. bullata* and *M. excelsa*, and between April 2021 and June 2021 for *L. obcordata* at each site) and dividing by the interval between measurements. A logarithm transformation was applied to Height growth rate values to stabilise the variance. Height growth rate was analysed using linear mixed models (LMMs), containing fungicide as fixed effects and 'mother tree' nested in 'species' in 'block' included as random effects. The LMMs for Height growth rate were constructed separately for each site and each species.

All LMMS and GLMMs were fitted by maximum likelihood via Template Model Builder (TMB), using automatic differentiation to estimate model gradients and the Laplace approximation for handling random effects (R package glmmTMB) (Brooks et al. 2017). For model validation, plots of Pearson residuals against the fitted values and versus each explanatory variable in the model were used. For all models, the significance of the fixed terms was assessed using a likelihood-ratio test for fixed effects in generalised linear mixed-effects models (Fox 2019; R package car), and the significant terms were followed up by applying a multiple-comparison procedure using Tukey contrast (Lenth 2019).

3 Results

3.1 Myrtle rust and other impacts on the six species

Four of the six species included in the trials were substantially affected by myrtle rust in both Auckland and Rotorua: *L. bullata, L. obcordata, M. excelsa* and *S. jambos* (Table 3, Figure 3). Some *L. scoparium* plants developed a low incidence (<0.5%) of myrtle rust on immature fruits (seed capsules) in the Auckland trial from November 2020 to March 2021 (Appendix 8.4, Figure 4). Eight out of 18 seedlings from two mother tree lines, 1430 and 1431, were affected. No leaf or stem infection was found on *L. scoparium* in Auckland and no infection at all was found on *L. scoparium* in Rotorua. No infection was reported from *K. robusta* at either site.

Myrtle rust was observed on young leaves, stems, flowers buds, flowers and immature fruits of *L. bullata* and *L. obcordata* (Figure 3). Myrtle rust dieback of new flush growth was observed in both trials within the first season of the seedlings being planted. New shoot growth occurred in the second season and this was accompanied by more dieback (Figures 3 and 6).

All species successfully established and began to produce new flush within 2–3 months after planting at both sites, except *L. obcordata*, for which establishment was poor with very little growth in the first season (Appendix 8.5). Over the two seasons, 44 out of the 129 *L. obcordata* planted in Rotorua did not grow, including 13 that died, and in Auckland 61 did not grow, including 18 that died. These plants were all excluded from data analysis. Height growth and new shoot production of *K. robusta* and *L. scoparium* was substantially greater than for the other species, with a particularly high growth rate for *K. robusta* in Auckland (Appendix 8.5). Frost damage to *M. excelsa* and *S. jambos* in Rotorua severely affected growth between July and September 2020, resulting, in some cases, in plant death. In Auckland, *M. excelsa* and *S. jambos* suffered minor shoot dieback from frosts during July and August 2020, but the plants quickly produced new flush in spring of the second season.

Table 3. Summary of observations on growth, fruiting, effects of myrtle rust (without fungicide) and other constraints on the six Myrtaceae species planted in the Rotorua and Auckland trials. Note that myrtle rust only developed on actively growing plants.

Species	Shoot growth	Flowering/fruiting	Myrtle rust	Insect damage	Frost damage
Lophomyrtus bullata	Vigorous growth, except in the coldest months	Approx. 25% of trees fruited in Rotorua during Season 2 (23/96), but minimal fruiting in Auckland	Severe leaf and stem infection; dieback from growing tip infection; flower and fruit infection and death	None	None
Lophomyrtus obcordata	Variable growth from poor establishment; long internodes on some plants in Season 2	Slight fruiting in Rotorua in Season 2 (4/118) but none in Auckland	Severe dieback from growing tip infection; leaf and stem infection present on vigorous plants; fruit infection and death	Scale insect on some poorly growing plants	None
Metrosideros excelsa	Juvenile foliage in Season 1 transitioning to adult foliage on some plants in Season 2. Discontinuous (rhythmic) growth on individual shoots	No flowering during the two seasons	Severe leaf infection; less stem infection; dieback, but not from direct infection of the growing tip	Pōhutukawa weevil damage in Season 2 in Auckland	Severe in Rotorua, including some plant death. Minor injury in Auckland
Syzygium jambos	Sometimes vigorous, but individual shoots showing discontinuous growth	No flowering during the two seasons	Severe leaf and stem infection and dieback at both sites	None	Severe damage from even mild frost at both sites
Leptospermum scoparium	Continuous, vigorous growth at both sites, declining during the coldest months	Profuse flowering in spring of Season 2 at both sites. No seeds released by the end of Season 2	No leaf or stem rust and no dieback. Slight fruit infection in Season 2, Auckland only	Leaf roller damage in Rotorua in Season 1	None
Kunzea robusta	Continuous growth and extremely vigorous, particularly in Auckland, where some new flush was present even in winter	Some flowering in spring of Season 2 and seeds released within 6 months	No rust observed at either site	Leaf roller damage in Rotorua in Season 1	None

3.2 Relationship between New flush leaves and Leaf severity

Although myrtle rust infection depends on new shoots being present, there were only weak positive correlations between Leaf severity and New flush leaves (Appendix 8.6). Stronger correlations were possibly obscured because the monthly monitoring interval was too long. During the periods of rapid disease increase, the time delay between infection of New flush leaves and appearance of symptoms (Leaf severity), would have been much less than 1 month. That time delay is the latent period and was around 7–10 days in Auckland and around 10–20 days in Rotorua (see Section 3.6, Weather monitoring and climatic risk, Figure 12). The Leaf severity/New flush leaves correlations were higher in Rotorua than Auckland for *L. bullata*, *L. obcordata and S. jambos* (Appendix 8.6, variables R1 and R5), where the latent period was longer and therefore more similar to the monthly monitoring interval. For *M. excelsa*, the latent period is slightly longer than in *Lophomyrtus* spp. and *S. jambos* and the correlations were slightly stronger in Auckland.

3.3 Relationship between Leaf severity and Stem disease

Leaf severity was highly positively correlated with stem infection for *Lophomyrtus bullata* (r=0.89), *L. obcordata* (r=0.84) and *S. jambos* (r=0.87) (Appendix 8.6). This means leaf area covered by rust or number of infected stems can be used to estimate disease severity for these species. For *M. excelsa*, Leaf severity and Stem disease showed no strong correlation. These considerations suggest that there is a slight tolerance of *M. excelsa* stems to myrtle rust. For this species, Leaf severity is a better indicator than Stem disease to estimate a plant's disease severity.

3.4 Seasonal variation in myrtle rust

There tended to be peaks and troughs in Leaf severity between late spring and late autmn. These reflected discrete epidemic cycles that occurred as new growth flush formed, became infected and died back. These were then followed by regrowth, reinfection and further dieback. In Rotorua, in both seasons, two epidemic peaks were evident for *L. bullata* and *S. jambos*, one in January and one in May. For Auckland, there was only one first season peak (May) and two second season peaks, one in November (*L. bullata*) or December (*S. jambos*) and the other in March/April (Figure 5). The Auckland trial was planted about a month later than the Rotorua trial, so both shoot growth and myrtle rust infection in the first season also commenced later, possibly accounting for only one first season severity peak in Auckland.

For *M. excelsa*, Leaf severity tended to increase more slowly than for *M. excelsa* and *S. jambos* and the only strong bimodal trend was in Auckland in the second season, with peaks in January and March (Figure 5). In Rotorua, in the second season, there was a single epidemic starting in January and peaking in May. Plant growth of *M. excelsa* in Rotorua in the second season was set back by the severe frost damage in the preceding winter (see Section 3.6, Weather monitoring and climatic risk). Disease severity for *M. excelsa* in the second season reached a similar magnitude to that in *L. bullata* and *S. jambos*.

For *L. obcordata*, severity was negligible during the first season and this was associated with poor plant establishment and lack of new shoot growth (Appendix 8.5). In the second season, especially in Auckland, some of the plants grew rapidly and became severely infected, giving bimodal severity peaks in December and March. Weak bimodal peaks also occurred in Rotorua in January and March in the second season.

Shoot dieback is a common symptom from myrtle rust infection of highly susceptible species. Shoot dieback was only monitored during the second season, between August 2020 and July 2021. For *L. bullata*, Total dieback (New dieback + Old dieback) increased markedly between November 2020 and January 2021 as myrtle rust infection progressed, with a faster rate of increase in Auckland than in Rotorua (Figure 6). During Season 2, severe New dieback from the initial epidemic was followed by subsequent episodes of new shoot growth, infection and further dieback. Total dieback therefore increased and continued to be severe for the rest of Season 2.

For *M. excelsa*, the rate of increase in dieback was much slower than for *L. bullata* and more-so in Rotorua, where shoot growth was delayed by frost damage during winter 2020. For *L. obcordata*, the myrtle rust dieback pattern in Auckland was similar to that for *L. bullata*, whereas in Rotorua very little dieback occurred because very little new shoot growth occurred. For *S. jambos* in Auckland, Total dieback was high over winter 2020 and declined during spring (September to November 2020) as new shoot growth occurred. It increased again in steps from December to May with the epidemic waves of

myrtle rust. In Rotorua, *S. jambos* dieback developed gradually as shoot growth and subsequent myrtle rust recovered from frost damage during winter.

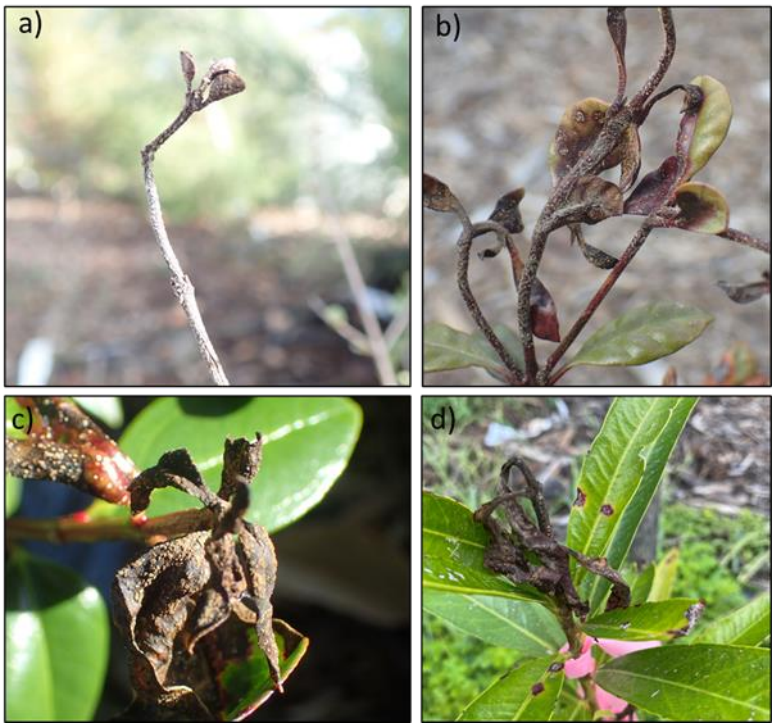

Figure 3. Branch dieback of new growth caused by myrtle rust infection on a) *Lophomyrtus obcordata*, b) *Lophomyrtus bullata*, c) *Metrosideros excelsa* and d) *Syzygium jambo*s.

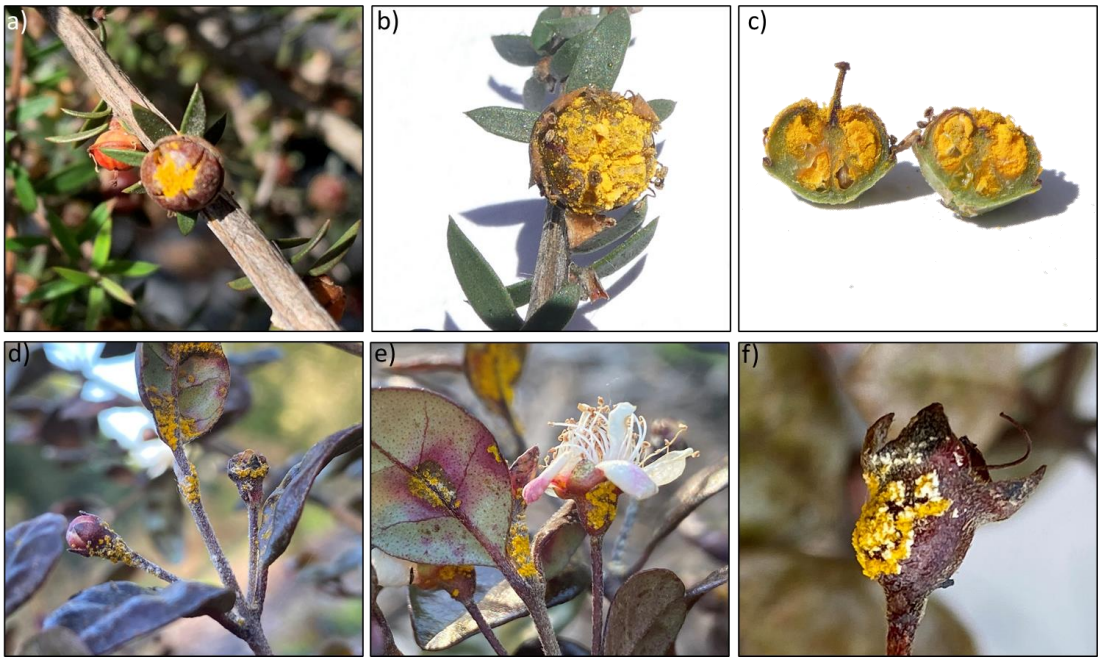

Figure 4. Myrtle rust infection on immature fruits of *Leptospermum scoparium* (a-c), inflorescence, flowers and immature fruits of *Lophomyrtus bullata* (d-f).

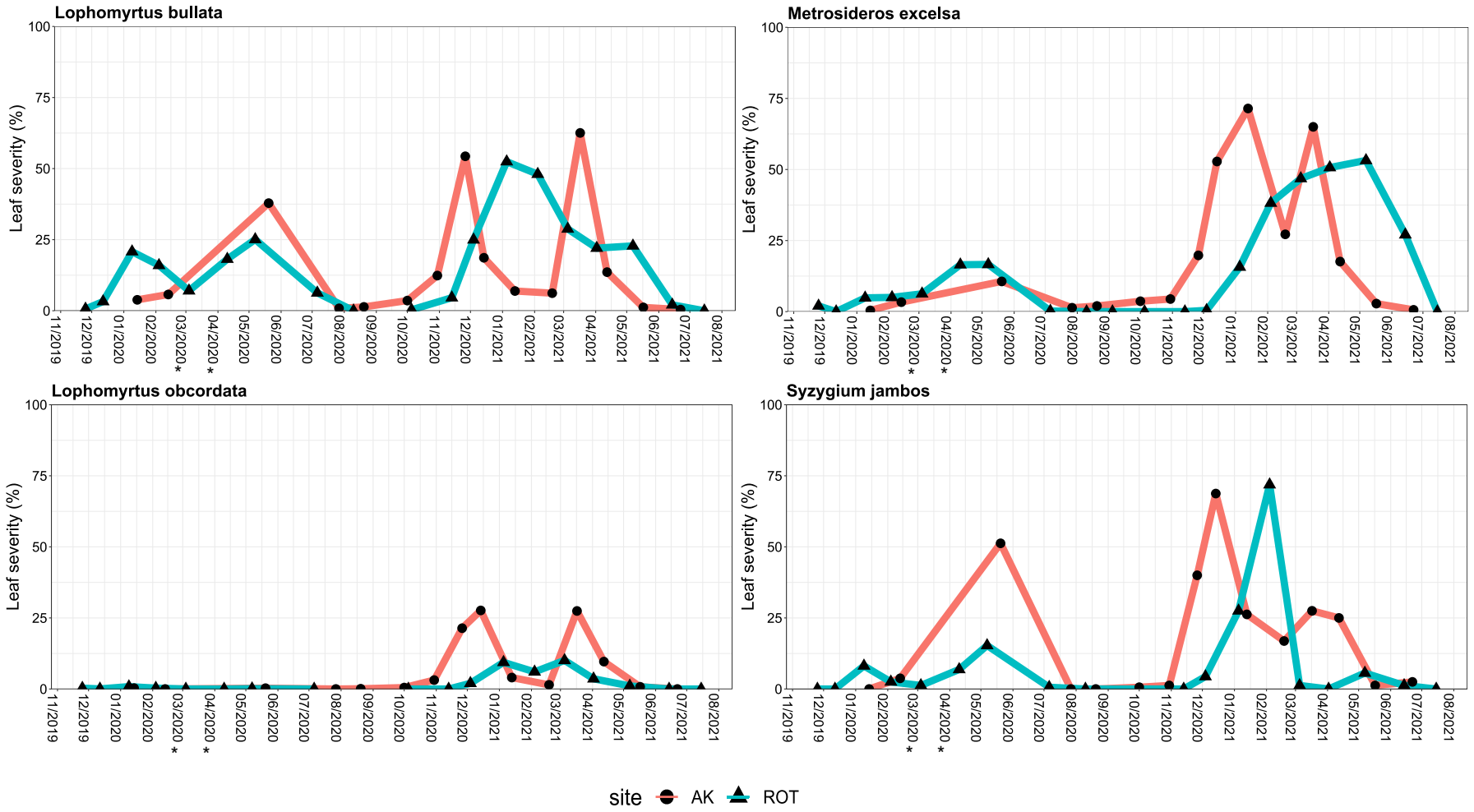

Figure 5. Monthly mean Leaf severity (% leaf area affected by myrtle rust) at each site for non-fungicide treated trees of the four species that showed myrtle rust leaf disease over two seasons. The asterisks show missing data from Auckland due to COVID-19 lockdown.

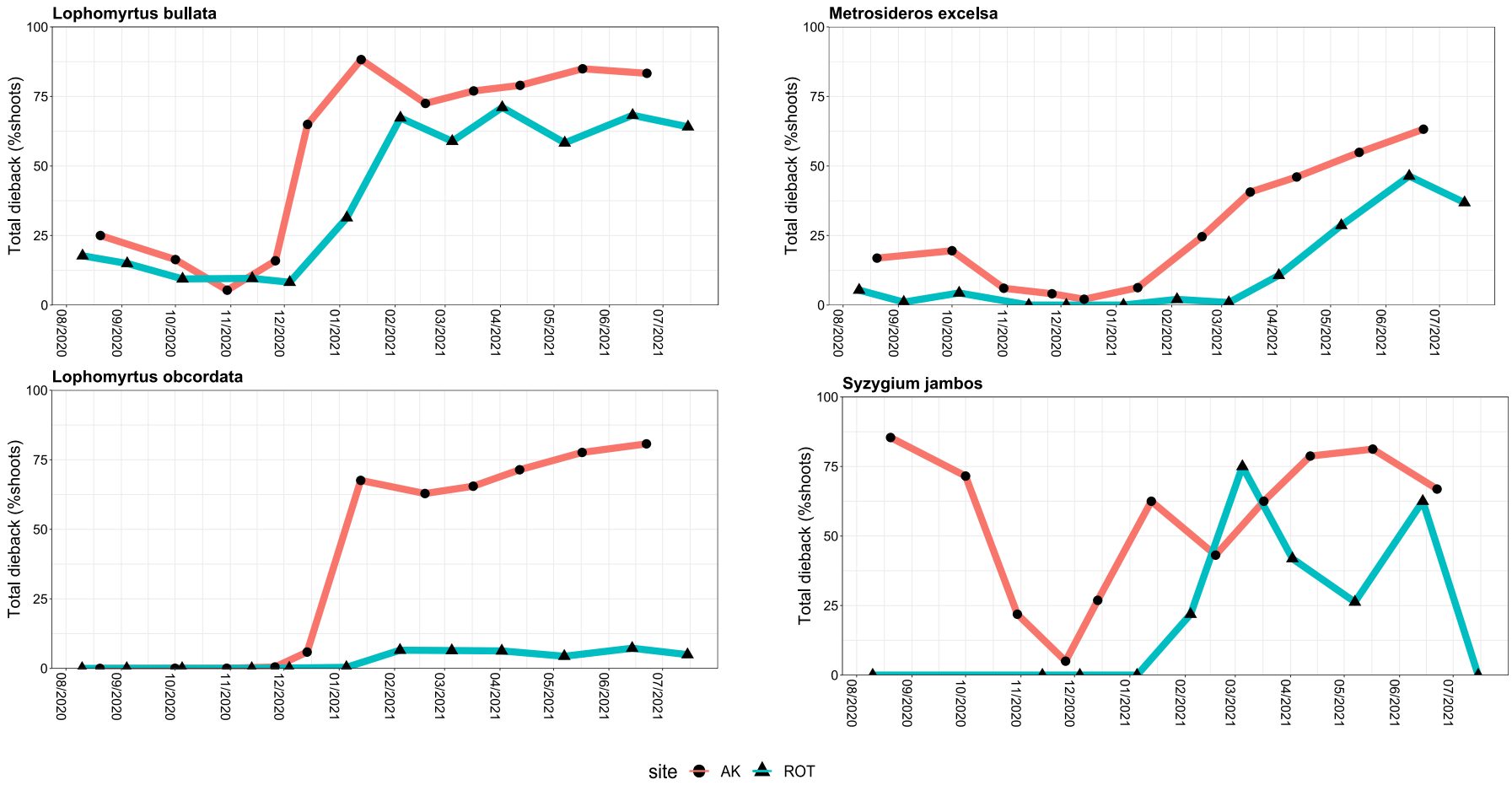

Figure 6. Monthly mean total dieback (new+old dieback) at each site for non-fungicide treated trees of the four species that showed myrtle rust dieback during Season 2.

3.5 Effect of fungicide treatment on myrtle rust

3.5.1 Impact myrtle rust on disease severity and new dieback

During Season 2, 10 fungicide applications were made to *L. bullata*, and *M. excelsa* in Rotorua and seven in Auckland (Appendix 8.3). These applications significantly reduced myrtle rust Leaf severity and New dieback and increased New flush leaves and Height growth rate at both sites (Figure 7, Appendix 8.7 and 8.8). For *L. bullata* in Auckland, substantial *A. psidii* leaf infection still developed on fungicide sprayed plants, but the amount of shoot dieback was substantially less and height growth noticeably greater compared with the non-sprayed plants.

For *L. obcordata*, three fungicide applications were made in Rotorua and two in Auckland (Appendix 8.3). There was no significant effect from the applications in Rotorua on New flush leaves, Leaf severity or New dieback. In Auckland, there was a significant reduction in New dieback, but no other significant effects (Figure 7; Appendix 8.9).

S. jambos was excluded from the statistical analysis because of the small number of plants (Appendix 8.4). Observed Leaf severity and New dieback of *S. jambos* were lower on fungicide-sprayed seedlings, while new flush and growth rate were higher at both sites compared with non-sprayed seedlings (Data not given).

The statistical analyses investigated the contribution of “species”, “fungicide”, “month (DOA)” and the interaction between these effects on the disease severity and related dieback in each trial.

In Rotorua, the application of fungicide and the species being sprayed had a significant effect on the variability of Leaf severity ($p < 0.001$; Appendix 8.7). Tukey’s multiple comparison test indicated Leaf severity was significantly higher for the control group (not-treated) of *L. bullata* and *M. excelsa* compared with the fungicide-treated trees ($p < 0.05$, Figure 7). There was no significant difference in Leaf severity between fungicide-treated *L. bullata* and *M. excelsa* ($p > 0.05$, Figure 7). New dieback was highly influenced by fungicide applications, species and timing of fungicide applications, hence there was a significant three-way interaction between “species”, “fungicide” and “month (DOA)” ($p < 0.05$; Appendix 8.7). There was significantly less New dieback on untreated trees of *M. excelsa* than *L. bullata* ($p < 0.05$; Figure 7), indicating possible resistance to dieback in this species. There was no significant effect of fungicide and month on Leaf severity and New dieback for *L. obcordata* growing in the Rotorua trial ($p > 0.05$; Appendix 8.9).

In Auckland, Leaf severity and New dieback varied significantly depending on the species, application of fungicide and timing of application ($p < 0.001$; Appendix 8). Leaf severity was significantly higher in the control group of *L. bullata* and *M. excelsa* compared with the fungicide-treated group ($p < 0.05$; Figure 7). The untreated group of *M. excelsa* showed significantly higher Leaf severity than the untreated *L. bullata* trees ($p < 0.05$; Figure 7). New dieback was significantly higher on untreated trees of *L. bullata* and *M. excelsa* compared with the fungicide-treated group ($p < 0.05$; Figure 7). Again, *M. excelsa* untreated trees expressed significantly less New dieback than *L. bullata* untreated group ($p < 0.05$; Figure 7). There was no significant interaction between “fungicide” and “month (DOA)” on disease severity for *L. obcordata*, but there was a marginally significant effect of fungicide application on New dieback ($p = 0.05$; Appendix 8.9).

3.5.2 Impact of myrtle rust on plant structure and growth rate

The repeated myrtle rust infection and related dieback had a substantial impact on the ability of individual plants to produce new flush leaves, particularly for some species.

In both trials, the application of fungicides had a protective effect on *L. bullata* and *M. excelsa*, but the protection was influenced by the species difference in timing of emergence of susceptible new flush, which explains the three-way interaction ($p < 0.01$, Appendix 8.7, Appendix 8.8). New flush leaves on *L. bullata* was significantly greater on the fungicide treated group compared with the controls ($p < 0.05$; Figure 7). Actively growing shoots did not significantly differ between control and treated trees of *M. excelsa* over the same time period ($p > 0.05$; Figure 7).

Height growth rate differed significantly between fungicide treated and controls for *L. bullata, M. excelsa* and *L. obcordata*. The exclusion of myrtle rust using fungicide application had a significant effect on Height growth rate of *L. bullata* in both trials ($p < 0.001$; Appendix 8.10), and on *M. excelsa* and *L. obcordata* in the Auckland trial only.

L. bullata trees treated with fungicides had a significantly higher growth rate (2.3 ± 0.3 cm/month in Rotorua; 6 ± 0.3 cm/month in Auckland) compared with control plants ($p < 0.05$; Figure 7; Appendix 8.11). *M. excelsa* treated with fungicides had significantly higher growth rate (4.1 ± 0.7 cm/month) compared with control plants in Auckland ($p < 0.05$; Figure 7; Appendix 8.11). *L. obcordata* treated with fungicides had significantly higher growth rate (1.2 ± 0.2 cm/month) compared with control plants in Auckland ($p < 0.05$; Figure 7; Appendix 8.9). No significant effect of fungicide treatment was observed on Height growth rate for *M. excelsa* and *L. obcordata* in Rotorua ($p > 0.05$; Figure 7; Appendix 8.11).

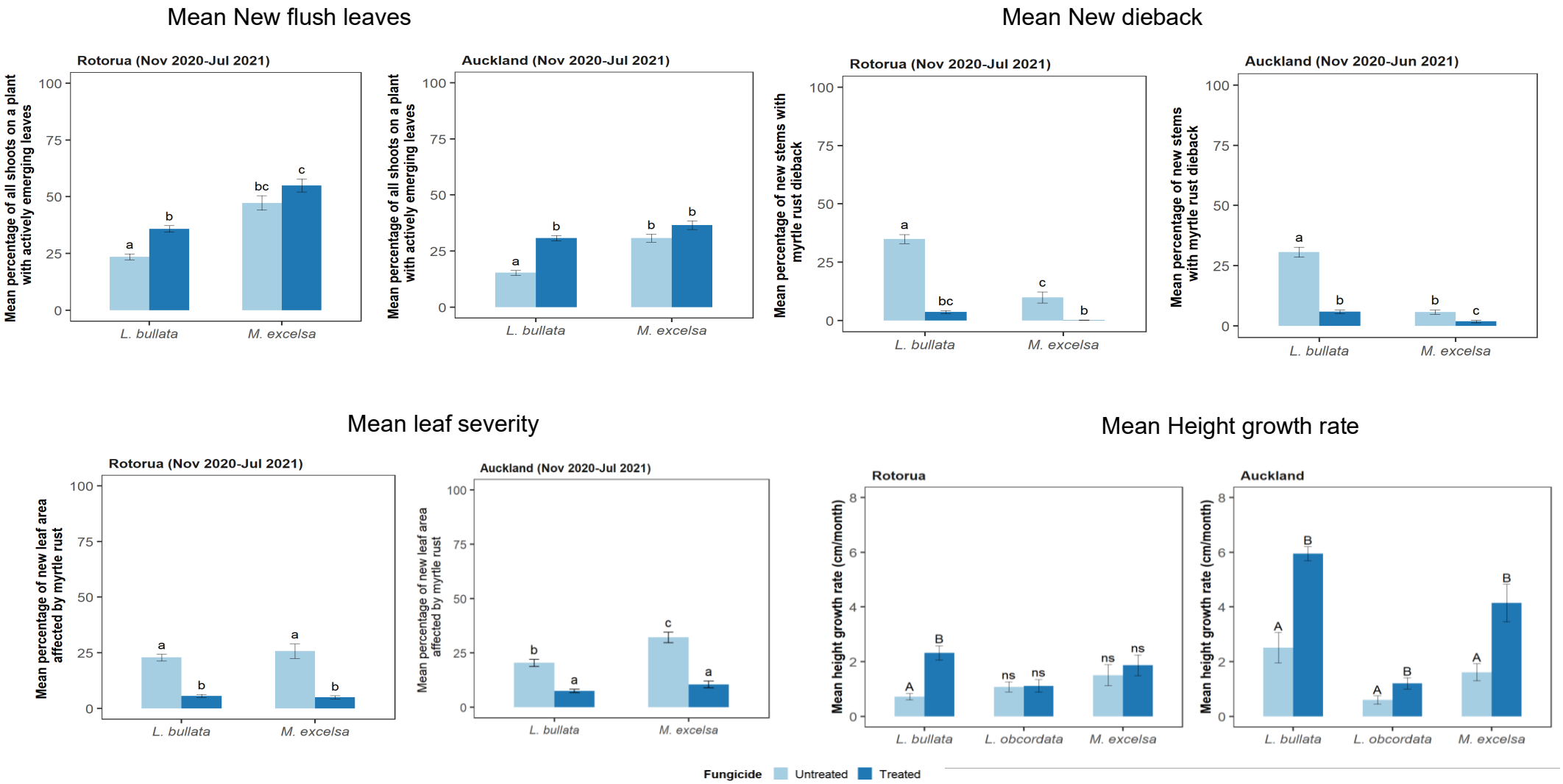

Figure 7. Multiple comparison tests for New flush leaves, New dieback, Leaf severity and Height growth rate per species and by fungicide treatment group ± standard errors (SE). The mean percentage across all time points from November 2020 to July 2021 is shown for *Lophomyrtus bullata*, *Metrosideros excelsa* and *Lophomyrtus obcordata* in Rotorua and Auckland. Different lower-case letters indicate statistically significant differences (Tukey contrast at α = 0.05).

3.6 Weather monitoring and climatic risk

3.6.1 Climatology in Rotorua and Auckland

There were substantial climatic differences during the 21-month study between Rotorua and Auckland, particularly for air temperature (Figure 8). These were associated with site differences in latitude and altitude, with Rotorua (38.158983° S, 176.263342° E, 290 m) being cooler than Auckland (36.891221°S, 174.727688° E, 38 m). Daily duration of high relative humidity (hours per day >85%) showed similar seasonal patterns at both sites. Rainfall was similar in total quantity (1958 mm Rotorua and 1765 mm Auckland), but differed in seasonal distribution between sites. Auckland was officially in drought during the study, so rainfall amounts were unusually low.

Monthly means of temperature, high relative humidity and rainfall (Figure 9) show that mean summer temperatures (December–February) in Rotorua were about 1°C cooler than in Auckland, but winter temperatures were 2–3°C cooler and winter frosts were more common in Rotorua (Table 4). High relative humidity duration tended to be 2–3 hours per day longer in Auckland in late autumn to early winter (April–June) in both years and also in January in both years.

Table 4. Occurrence of air frost 1.5 m above ground at Rotorua and Auckland trial sites from 1 November 2019 to 31 July 2021.

		Rotorua	Auckland
Season	Date	Min. temp (°C)	Min. temp (°C)
2019-20	2-May-20	−0.2	
	22-May-20	−0.4	
	2-Jul-20	−0.8	
	3-Jul-20	−0.8	−0.6
	4-Jul-20	−0.2	
	10-Jul-20	−1.1	−0.6
	27-Jul-20	−0.6	
	9-Aug-20	−0.2	
	16-Aug-20		−0.9
	17-Aug-20	−0.7	
	28-Aug-20	−0.1	
	Total	10	3
2020-21	28-May-21	−0.2	
	30-Jun-21	−0.3	−1.1
	4-Jul-21		−0.5
	5-Jul-21	−0.6	−0.2
	14-Jul-21		−1.6
	Total	3	4

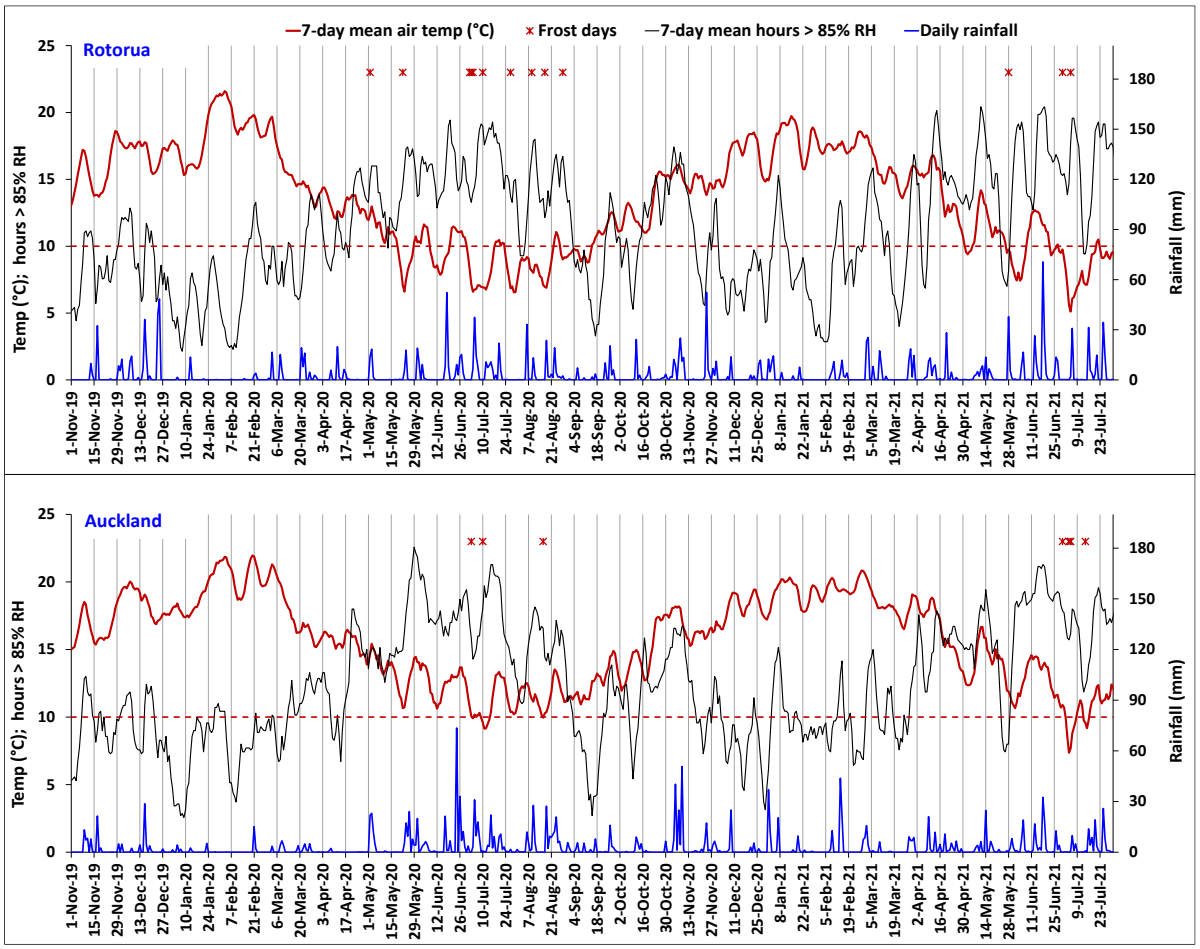

Figure 8. Air temperature, frost days, duration of high relative humidity (RH >85%) and rainfall associated with myrtle rust at the Rotorua and Auckland trial sites between November 2019 and July 2021. Temperature and RH are 7-day running means and rainfall is daily total. The red dashed 10°C line indicates the approximate temperature around which myrtle rust development becomes very slow. Frost days refer to air frosts (minimum air temperature <0°C, 1.5 m above ground).

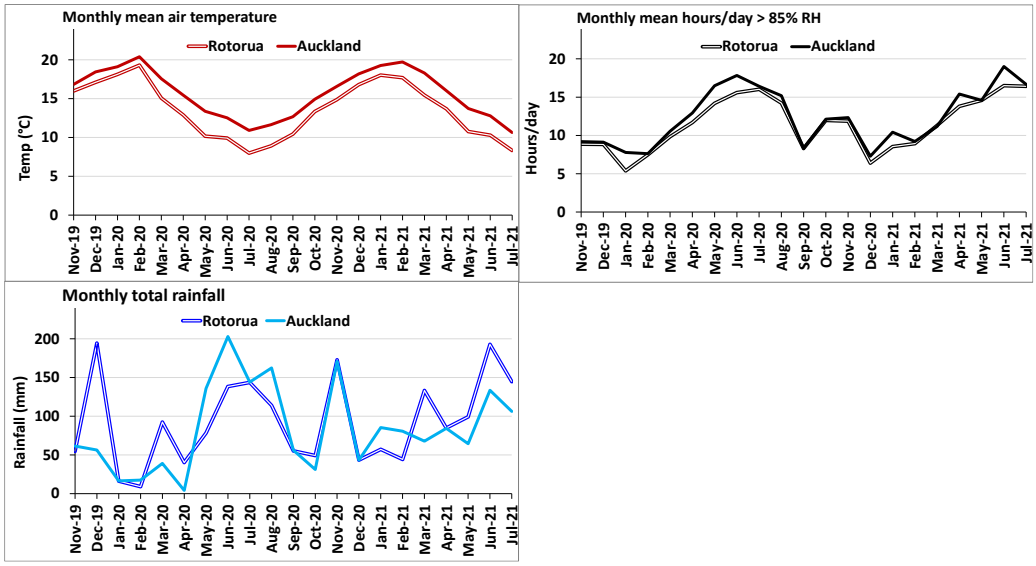

Figure 9. Monthly means of air temperature, hours/day with relative humidity (RH) >85% and total rainfall at the at Rotorua Auckland field susceptibility trial sites between November 2019 and July 2021.

3.6.2 Modelled myrtle rust risk in Rotorua and Auckland

Infection risk was substantially greater in Auckland than Rotorua in the moderate, high and very high infection risk categories (Figure 10). In Auckland, there were more months with greater infection risk than in Rotorua, giving Auckland a longer season for myrtle rust infection, particularly during late autumn (April–May) and early spring (September–October). Infection risk was lowest at both sites in late winter and the beginning of spring (July–early September). Appendix 8.12 gives daily infection risk values.

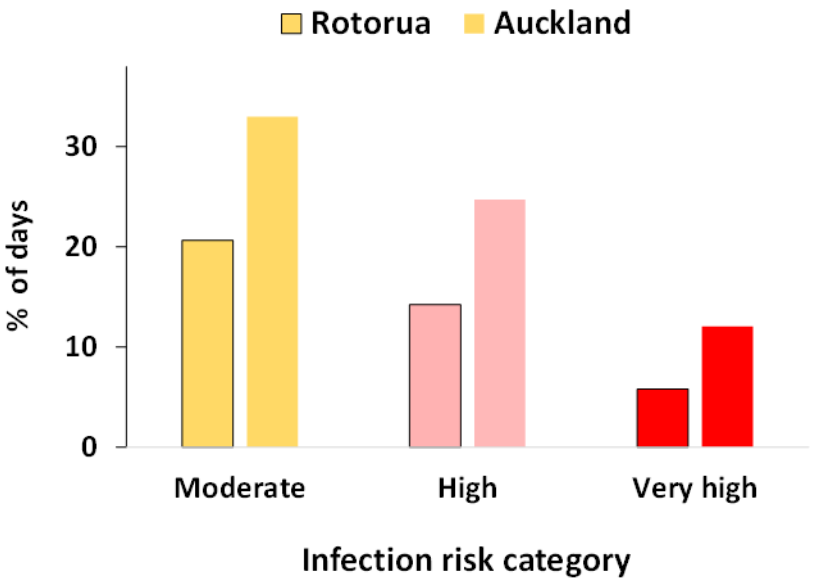

Figure 10. Percentage of days with myrtle rust infection risk in moderate, high and very high risk categories at the Rotorua and Auckland trial sites between 1 November 2019 and 31 July 2021 (639 days).

Latent period is the time from infection to production of new spores and has a key influence on seasonal myrtle rust development because it determines the rate at which multiplication can occur after initial infection. Latent period is also important in relation to *A. psidii* overwintering because at mean temperatures <10–12°C, *A. psidii* remains latent for extended periods.

Latent period is primarily influenced by temperature (Figure 11), but host species is also important (Beresford et al. 2020). At optimum temperatures of 24–28°C, the latent period is 6–7 days for *M. excelsa*, 5–6 days for *Lophomyrtus spp.*, and 4–5 days for *S. jambos*. Below 20°C, latent period increases rapidly with decreasing temperature and, in *Lophomyrtus* and *S. jambos*, at temperatures below 10–12°C *A. psidii* is largely inactive, whereas in *M. excelsa*, it is inactive below 13–15°C. This difference between hosts is probably because of genetic susceptibility differences and host physiological adaptation to temperature. Because low temperature slows *A. psidii* development, low winter temperatures in cooler climates (southern New Zealand and higher altitude) allow *A. psidii* to overwinter as latent infection until seasonal warming occurs in spring.

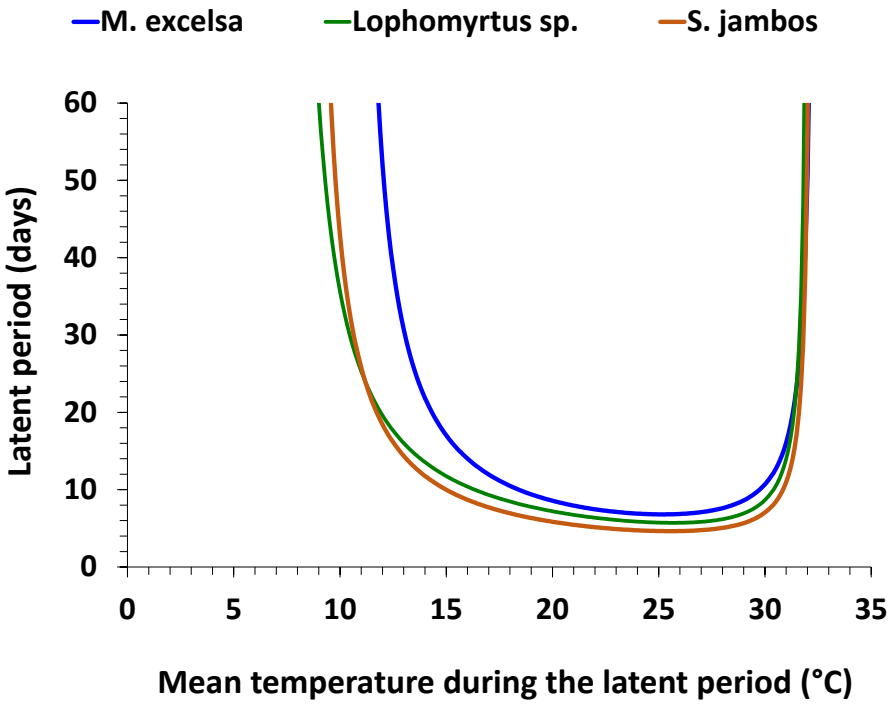

Figure 11. Latent period of *Austropuccinia psidii* in relation to temperature on three susceptible host species (*Metrosideros excelsa*, *Lophomyrtus* sp., (*L. bullata* x *L. obcordata* hybrid) and *Syzygium jambos*). From Beresford et al. (2020).

For the 21 months of this study, the above latent period information was processed using field temperatures as input to the Myrtle Rust Process Model to calculate daily instantaneous latent period for *M. excelsa* and *Lophomyrtus* spp. in Rotorua and Auckland.

In concordance with temperature trends, Auckland had shorter latent periods and therefore higher latent period risk than Rotorua. *Lophomyrtus* spp. had higher latent period risk than *M. excelsa*, reflecting more rapid latent development at lower temperatures in this host (Figure 12).

Predicted latent period became very long in winter in Rotorua, especially for *M. excelsa*. In Auckland, minimum latent period in summer (December–February) was consistently closer to the minimum value compared with Rotorua, where values increased during cooler periods.

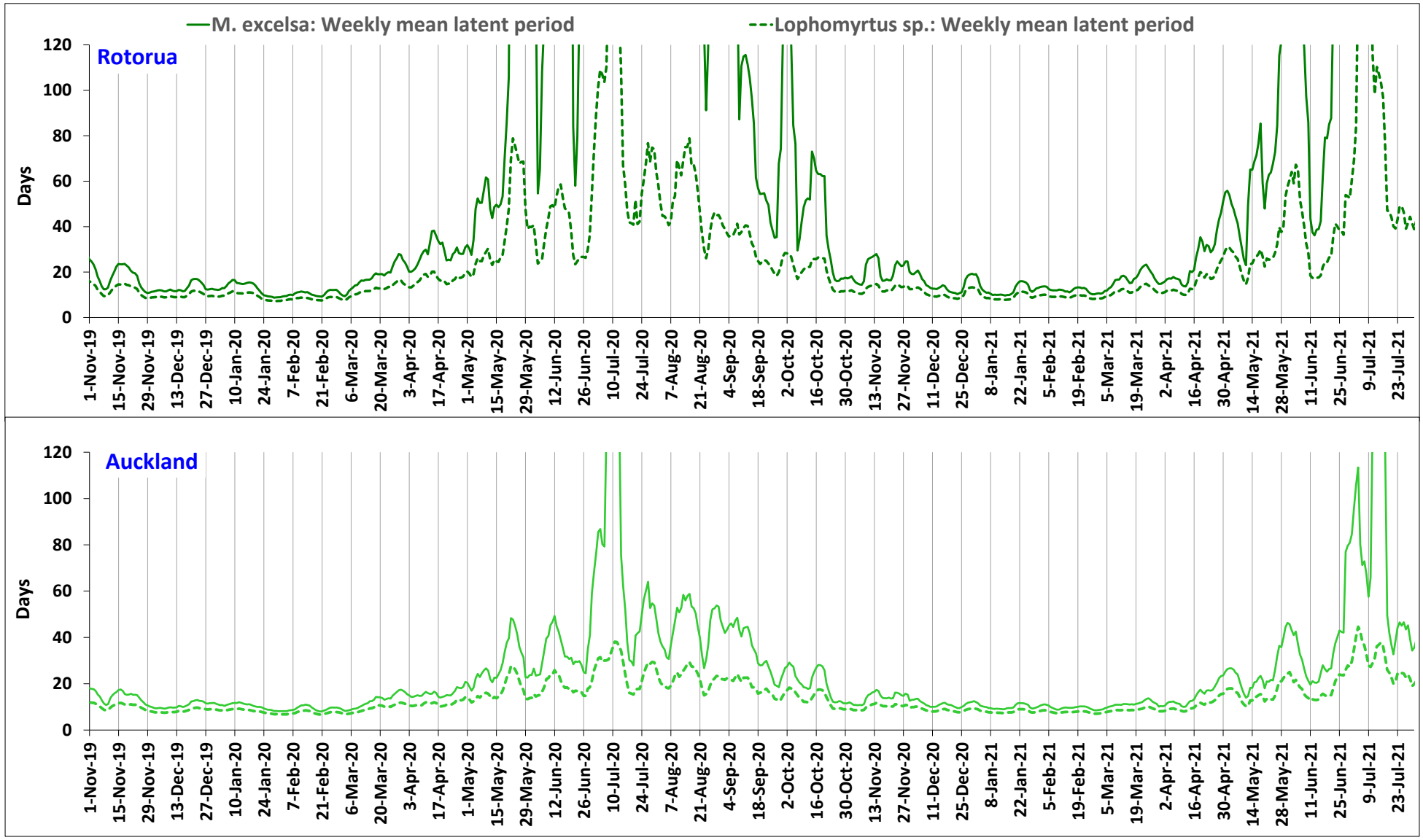

Figure 12. Seasonal variation in modelled myrtle rust latent period for the Auckland and Rotorua trial sites between November 2019 and July 2021. Latent period was calculated as daily instantaneous values from air temperature (Beresford et al. 2020) and is presented here as weekly means.

The tendency for *A. psidii* to overwinter as latent infection was expressed as the number of latent periods completed in a month. If this variable is less than 1 latent period per month, then the pathogen can be considered to be overwintering as latent infection.

The modelling predicts that *A. psidii* overwintered as latent infection within infected *M. excelsa* tissues between May and September in Rotorua (Figure 13), whereas this would have only happened during July and August in Auckland. For *Lophomyrtus* spp. in Rotorua, there would have been some tendency for latent overwintering in July and August, but in Auckland latent overwintering would not have occurred at all.

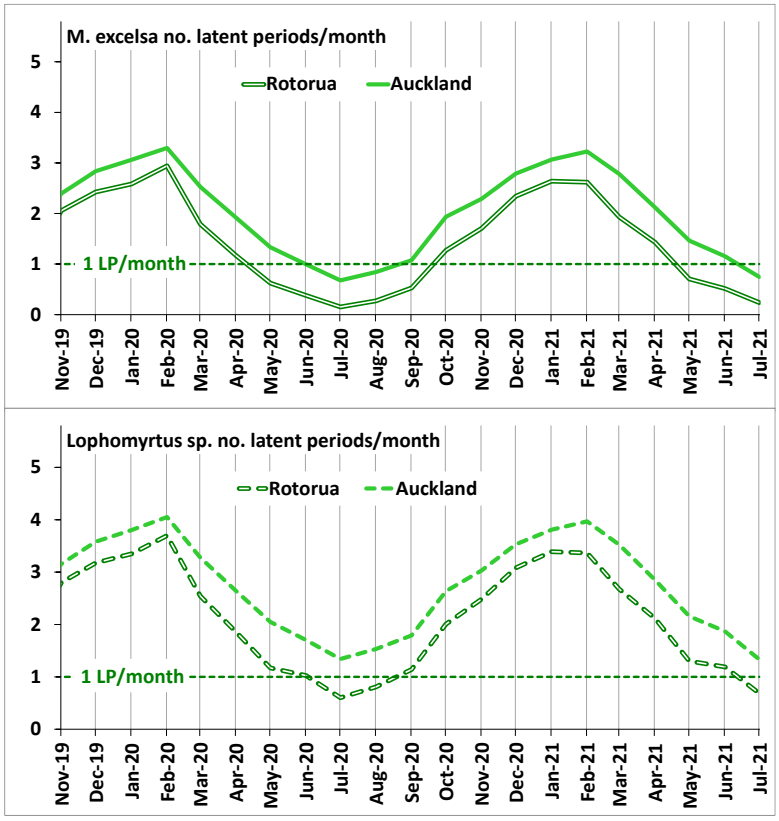

Figure 13. Number of latent periods (LPs) completed per month between November 2019 and July 2021 at the Rotorua and Auckland trial sites for *Metrosideros excelsa* and *Lophomyrtus* spp. Below the dashed line (1 LP/ month), *Austropuccinia psidii* is considered to be overwintering as latent infection. LPs were calculated from daily temperature in a continuous sequence over the duration of the study. LPs/month values are normalised to 30 days/month.

4 Discussion

4.1 Susceptibility of species in the field trials

Our study has demonstrated that different Myrtaceae species have different responses and degrees of vulnerability to *A. psidii* infection under natural field conditions. Four out of the six species were severely rust affected at both sites (*L. bullata, L. obcordata, M. excelsa* and *S. jambos*). The other two species (*L. scoparium* and *K. robusta*) were not affected at all, except for a small incidence of fruit infection observed on *L. scoparium* in Auckland in the second season. Myrtle rust effects on non-fungicide treated plants of each species are summarised as follows:

- *Lophomyrtus bullata*. Infected trees consistently showed severe leaf, stem and growing tip infections that led to significant shoot dieback. This caused debilitation of plant growth over 1–2 months during summer epidemics and repeated infection prevented recovery. When present, inflorescences, flowers and fruits were also infected, reducing the chances for this species to reproduce.
- *Lophomyrtus obcordata*. Information obtained was incomplete because of the poor plant establishment and limited shoot growth in most mother plant lines. Leaf and stem infection were observed on vigorous plants only. When present, fruits were infected in Rotorua. Increased growth in Auckland in Season 2 was associated with severe growing tip dieback.
- *Metrosideros excelsa*. Severe leaf infection but minor stem infection at both sites. Severe dieback developed gradually as leaves died, rather than from direct infection of the growing tip, as occurs in *Lophomyrtus* spp. and *S. jambos*.
- *Syzygium jambos*. Severe leaf and stem infection, growing tip infection and extensive dieback at both sites.
- *Leptospermum scoparium*. No leaf or stem infection and no dieback. A few occurrences of fruit infection in Season 2 in Auckland only.
- *Kunzea robusta*. No infection on any plant organs observed at either site.

This study also provided insights into species genetic susceptibility. *M. excelsa* showed a slower rate of severity increase, less stem infection, little growing tip infection and substantially less shoot dieback compared with *L. bullata* under the same inoculum and environmental conditions. Genetic mechanisms contributing to greater resistance in *M. excelsa* could include morphologial features, such as the cataphylls that protect the emerging leaves in adult foliage of *M. excelsa* (Appendix 8.2 d). In addition, *M. excelsa* has a slightly longer latent period than *L. bullata* and *S. jambos* (Beresford et al. 2020), which is an indication of a degree of genetic resistance.

While *L. obcordata* was more severely affected by myrtle rust during the second season of observation, its slow establishment meant that further field data are needed before drawing strong conclusions about this species' field susceptibility. The poor plant establishment of *L. obcordata* at both sites may have been due to exposure of the young plants to wind, water stress and intense sunlight in a species that is more adapted to a forest understory environment. In addition, lower soil fertility on the sandy pumice soil in Rotorua may have affected plant vigour compared with the volcanic loam soil in Auckland.

4.2 Comparison with artificial inoculation screening

Many studies have evaluated the susceptibility of Myrtaceae species to different strains of *A. psidii* using artificial inoculation methods (Zauza et al. 2010; Pegg et al. 2014; Silva et al. 2014), including for New Zealand species (Smith et al. 2020; Soewarto et al. 2021). What is often overlooked is how results from controlled conditions translate back to field situations. Although complete results from the artificial inoculation study of Smith et al. (2020) are not available at the time of writing this report (October 2021), we are able to make some comparisons between the field trial data and the artificial inoculation data that are available (Appendix 8.13). A summary of findings from the artificial inoculations is as follows:

- *Leptospermum scoparium*. No data were available for the *L. scoparium* families planted in the field trials. However, other data from (Smith et al. 2020) indicate that *L. scoparium* was highly susceptible as small seedlings under artificial inoculation. Leaf and stem infection were reported from 62% of 1758 seedlings in 132 seed families. Leaf or stem resistance was found in about one-quarter of the plants. Symptoms were also reported on flower buds and open flowers in a small number of plants that flowered as young seedlings.
- *Kunzea robusta* and *K. linearis*. These two *Kunzea* spp. were analysed together in Smith et al. (2020) because of the limited number of seed families and seedlings. Low susceptibility to myrtle rust leaf and stem infection was observed in most of the 362 plants tested from 28 seed families.
- *Metrosideros excelsa*. High leaf susceptibility was observed in the 570 plants from 31 seed families under artificial inoculation. Only one resistant plant was found.
- *Lophomyrtus bullata*. High leaf susceptibility was observed in all 56 plants from three seed families. No resistant plants were found.
- *Lophomyrtus obcordata.* Only one family and five individual plants were tested under artificial inoculation. All five individuals were highly susceptible with infection localised only on the leaves. Under field conditions, we tested 16 families (different from the ones tested under artificial inoculation) and, for plants that were actively growing, found high susceptibility with infection on both leaves, stems, immature fruits with shoot dieback.

The susceptibility of species in the artificial inoculations broadly agreed with the field trial findings, except for the following points:

- In the artificial inoculations, *L. scoparium* appeared to have substantial leaf and stem susceptibility that was not seen in the field trials. Myrtle rust infection of *L. scoparium* has seldom been reported in the New Zealand natural environment, except for a few observations during the initial incursion response and the fruit infection observed in the Auckland field trial in this study.
- The field trials suggested that *L. obcordata* had higher leaf than stem susceptibility.
- Artificial inoculations suggested that *M. excelsa* had similarly high susceptibility to *L. bullata*, but the field trials showed lower susceptibility in *M. excelsa*, particularly in relation to stem infection, dieback and rate of disease progression.

The field trial disease assessment and epidemiological data suggest that species genetic susceptibility is more complex than can be characterised using artificial inoculation of young seedlings (4 months old in Smith et al. 2020). For example, there is much anecdotal evidence that young *M. excelsa* plants in nurseries and epicormic shoots at the base of larger trees become infected, but infection in the

upper canopy of mature trees in the natural environment is not being reported. An understanding of how host development and anatomy influence susceptibility for each species would be necessary to fully characterise genetic susceptibility/resistance, but this would probably have to be done using field evaluations rather than controlled environment inoculations.

4.3 Use of fungicides to limit myrtle rust infection

Previous studies investigated the effectiveness of fungicides against myrtle rust on New Zealand susceptible species (Pathan et al. 2020; Adusei-Fosu et al. 2021). These studies were under controlled conditions and used artificial inoculation to evaluate the efficacy of a range of fungicide mixtures to control *A. psidii* infection. The aim of our disease-exclusion trial was to quantify the effect of repeated *A. psidii* infection on the susceptible New Zealand Myrtaceae under natural field conditions. While three different active ingredients were used in rotation, the trial was not designed to test individual fungicides against myrtle rust. Therefore, the three fungicides were regarded as one single treatment.

The fungicide applications unequivocally reduced myrtle rust impacts at both sites and this was reflected in reductions in Leaf severity, Stem disease and, particularly, shoot dieback. This substantially improved plant growth by limiting the impact of myrtle rust on new flush and Height growth rate was significantly increased in fungicide treated *L. bullata* plants at both sites and in *M. excelsa* and *L. obcordata* in Auckland only. Effects of *A. psidii* in reducing growth in susceptible Australian species have been reported during glasshouse experiments (Fernandez Winzer et al. 2020, 2018).

The statistical significance of fungicide effects in each trial varied with plant species and month of assessment, possibly due to differences in timing of plant growth and rust development in the treated species (*L. bullata*, *L. obcordata*, *M. excelsa* and *S. jambos)*.

Our statistical analyses also indicated that the timing of fungicide application differentially affected disease severity. The magnitude of the fungicide effect was greatest when plant growth and myrtle rust infection were most active. Therefore, controlling myrtle rust on susceptible species in the field was very challenging and only partial control was achieved at both sites. Disease control was also limited because spraying started after myrtle rust had increased substantially and there were large numbers of spores produced by unsprayed infected plants adjacent to the sprayed plants. Fungicides can reduce infection and control disease if applied early in a disease epidemic; however, they cannot stop an epidemic that is well underway, as occurred in these trials. When substantial disease has developed, latent infection within plants may escape the effects of fungicide and imperfect fungicide deposition means some infection sites remain unprotected.

There were no significant fungicide effects for *L. obcordata* in Rotorua, probably because both growth and myrtle rust were limited by site factors. No significant fungicide effect was recorded on the *M. excelsa* growth in Rotorua because of repeated episodes of hard winter frost (Table 4), which particularly affected tender new growth, and sometimes killed the plants.

4.4 Myrtle rust epidemiology

These trials provided a unique opportunity to assess the impacts of myrtle rust in two climatically different regions and they provided new epidemiological insights into how differences in seasonal growth and rust development affect myrtle rust impacts on different hosts. Use of existing information on climatic requirements for *A. psidii* infection and latent period helped us to interpret the mechanisms behind the observed species differences in myrtle rust development at the trial sites.

The conditions favourable for myrtle rust infection are a minimum of 6–8 hours of surface wetness or high relative humidity and temperatures of 12–20°C (Beresford et al. 2018). The pathogen is adapted to night time infection, with diurnal temperature and humidity fluctuations providing wetness, and spore germination being inhibited by solar radiation during the day. Rainfall is correlated with myrtle rust infection through its effect in increasing ambient relative humidity and wetness duration. *A. psidii* is best adapted to the warm temperatures of the sub-tropics where it originated and in New Zealand, temperatures are generally below optimum. Temperature therefore critically determines where and when myrtle rust can develop in New Zealand.

In these trials, seasonal temperature was the key driver for both plant growth and myrtle rust development. Monthly average temperatures were 1–3°C cooler in Rotorua than in Auckland, whereas monthly average relative humidity and total rainfall were quite similar at both sites. The greater infection risk in Auckland compared with Rotorua therefore appeared to be driven by temperature, more than relative humidity. Under warmer conditions, as in Auckland, myrtle rust epidemics tended to occur in two waves each season:

1. Shoot growth began in early spring, followed by increasing rust severity on new shoots from late spring, which peaked in mid-summer and caused dieback of all the new growth. At the end of the first wave, in January, myrtle rust symptoms became very difficult to find.

2. Shoot growth began again in late summer (February) and a second epidemic developed during early autumn (March/April). This wave either again killed all the shoots before winter, or, if temperatures were cooler, as in Rotorua, disease increase rate declined. Depending on species, severity of dieback and autumn temperatures, some further new growth was possible before winter. By late autumn, temperatures were becoming too cool for new rust infection and existing symptoms were diminished by abscission of infected leaves and new growth.

Under the cooler conditions in Rotorua, double waves of infection were less pronounced or did not occur. Going into winter, there were very few symptoms and *A. psidii* was presumed to be present predominantly as latent infection. Latent period modelling suggested temperatures were cool enough for latent overwintering in *M. excelsa* in Rotorua for 5–6 months (May to September/October) and for 2–3 months (June to September) in Auckland. Frosts killed many of the *M. excelsa* and *S. jambos* shoots in Rotorua during the first winter, and therefore myrtle rust did not survive within the plants. For *L. bullata* in Rotorua, latent overwintering was likely to occur for 2–3 months (June to August) and would not have occurred at all in Auckland. In Auckland, some new pustules were observed during warmer periods during the winter months.

5 Conclusions

- Four out of the six species were severely affected by myrtle rust at both sites: *L. bullata*, *L. obcordata*, *M. excelsa* and *S. jambos*.
- Of the New Zealand native species, *L. bullata* showed the greatest susceptibility, which was comparable with that of *S. jambos.*
- *M. excelsa* showed a slower rate of disease increase, less stem infection and substantially less shoot dieback compared with *L. bullata* under the same inoculum and environmental conditions and this species appears to have a degree of genetic resistance to *A. psidii*.
- A small incidence of fruit infection was observed on *L. scoparium* (mānuka) in Auckland. This discovery is the first report of myrtle rust infecting reproductive structures of mānuka in New Zealand. It is concerning because it indicates susceptibility of flowers/fruit in some mānuka genotypes that could potentially be a threat to the mānuka honey industry and to natural regeneration of mānuka.
- No myrtle rust was detected on *K. robusta* under field conditions in these trials.
- These ex-situ plantings were made up of a small number of seed families and may not represent the full range of vulnerabilities to *A. psidii* present in these species nationwide.
- The disease assessment variables that best reflected myrtle rust effects were: Leaf severity, for intensity of *A. psidii* infection, Total dieback, for effects on plant growth and Flower/fruit disease for effects on reproductive potential. Stem disease was useful for understanding finer details about how myrtle rust impacts different species.
- Species susceptibility screening using artificial inoculation of seedlings under controlled environments cannot always predict field disease outcomes because of the interactions that occur in the natural environment between seasonal growth, flowering/fruiting phenology, pathogen inoculum, climatic conditions and changes in host anatomy and physiology with plant age.
- Fungicide applications were highly effective in reducing myrtle rust impacts in the Rotorua and Auckland trials. The greatest measured benefits were a reduction in shoot dieback, increased plant height and increased fruit survival.
- The timing of fungicide applications in relation to species differences in new flush shoot emergence is an important consideration to improve plant protection against myrtle rust.
- Seasonal temperature appeared to be the key driver for both plant growth and myrtle rust development in the Rotorua and Auckland trials.
- Climate change is likely to exacerbate the impacts of myrtle rust in New Zealand in the future because rising temperatures and shifting rainfall patterns will increase rates of *A. psidii* infection and multiplication.

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8 Appendix

8.1 List of species, families and provenance

AKL = Auckland; ROT = Rotorua.

Species	Provenance	Family	n (AKL)	n (ROT)	Tested under artificial inoculation
Kunzea robusta	Auckland	1250	8	8	yes
		1249	8	8	yes
		1248	8	8	yes
		1247	8	8	yes
		1165	8	8	yes
Leptospermum scoparium	Auckland	1426	10	8	yes
		1427	8	8	yes
		1428	6	8	yes
		1429	5	8	yes
	Rotorua	1430	9	8	yes
		1431	9	8	yes
		1432	9	8	yes
		1434	8	8	yes
Lophomyrtus bullata	Rotorua	A1	16	16	Three of these families were tested under artificial inoculation
		B	16	16	
		C	16	16	
		D	16	16	
		E	16	16	
		F2	16	16	
Lophomyrtus obcordata	Auckland	M17124	6	8	
		M17125	6	8	
		M17126	8	8	
		M17127	7	8	
		M17128	9	8	
		M17160	9	8	
		M17161	8	8	
	Rotorua	M17060	8	8	
		M17134	7	10	
		M17135	7	8	
		M17136	8	8	
		M17137	14	8	
		M17138	8	8	
		M17155	9	8	
		M17156	7	7	
		M17157	7	8	
Metrosideros excelsa	Auckland	1176	8	8	yes
		1175	8	7	yes
		1285	8	8	yes
		1284	8	8	yes
		1292	8	8	yes
		1291	8	8	yes
Syzygium jambos	Auckland	SJ_WAI	8	8	
Total			384	384	

8.2 Recording guide for phenological stages

Recording guide for presence of phenological stages (vegetative and reproductive) on five native Myrtaceae

Figure A.1. *Lophomyrtus bullata*: a) new expanding leaves and internodes present from just visible to fully expanded. b) flower bud; c) fully opened flowers; d-e) immature fruits; f) fully matured fruit.

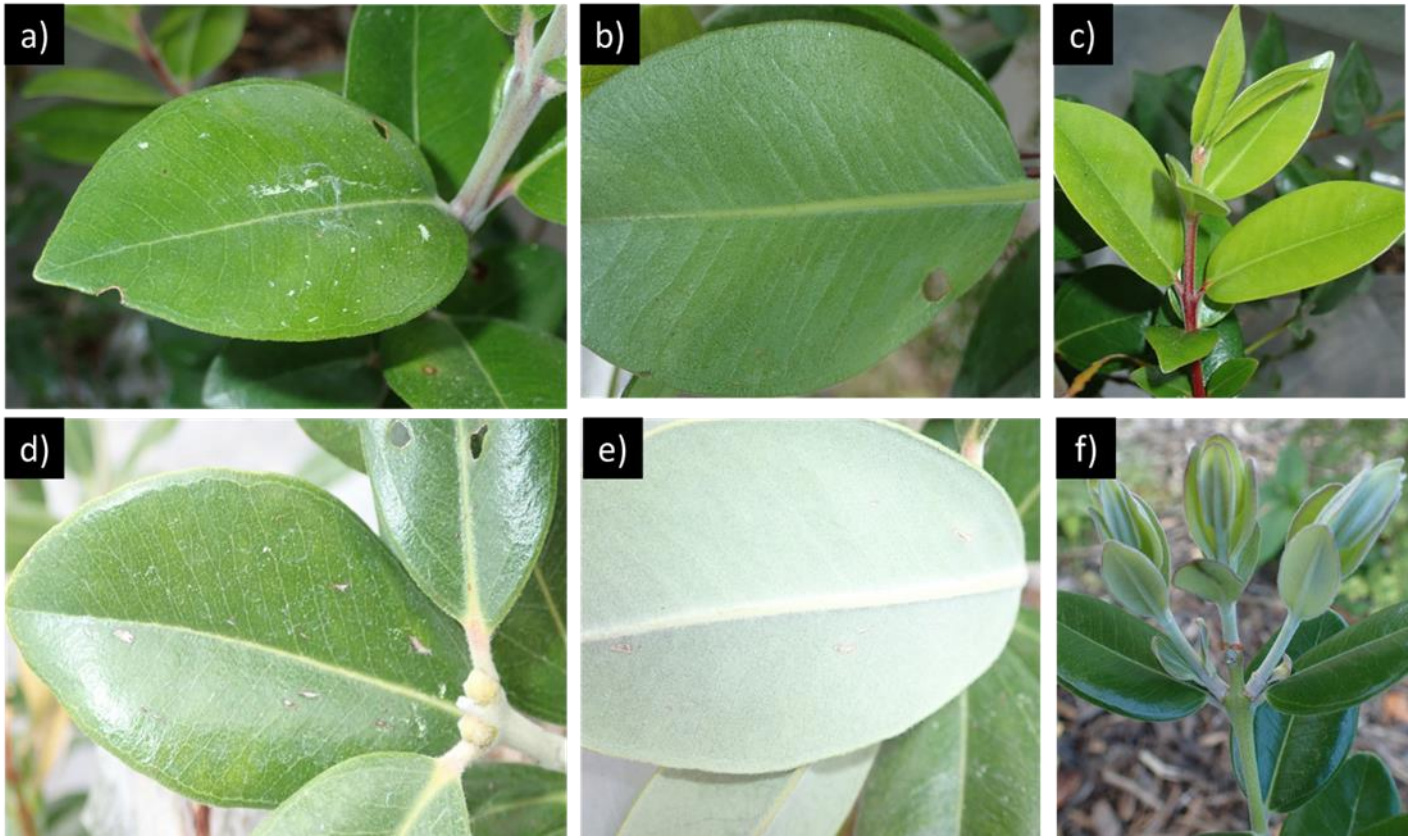


Figure A.2. *Metrosideros excelsa*: New expanding leaves and internodes present from just visible to fully expanded.
Abaxial view of juvenile leaf (a) with smooth adaxial surface (b-c).
Abaxial view of adult leaf form (d) with hairy adaxial surface (e-f).

Figure A.3. *Lophomyrtus obcordata:*

a-b) new expanding leaves and internodes present (from just visible to fully expanded showed by white arrows);
c) flower bud; d) flower; e) immature fruit.

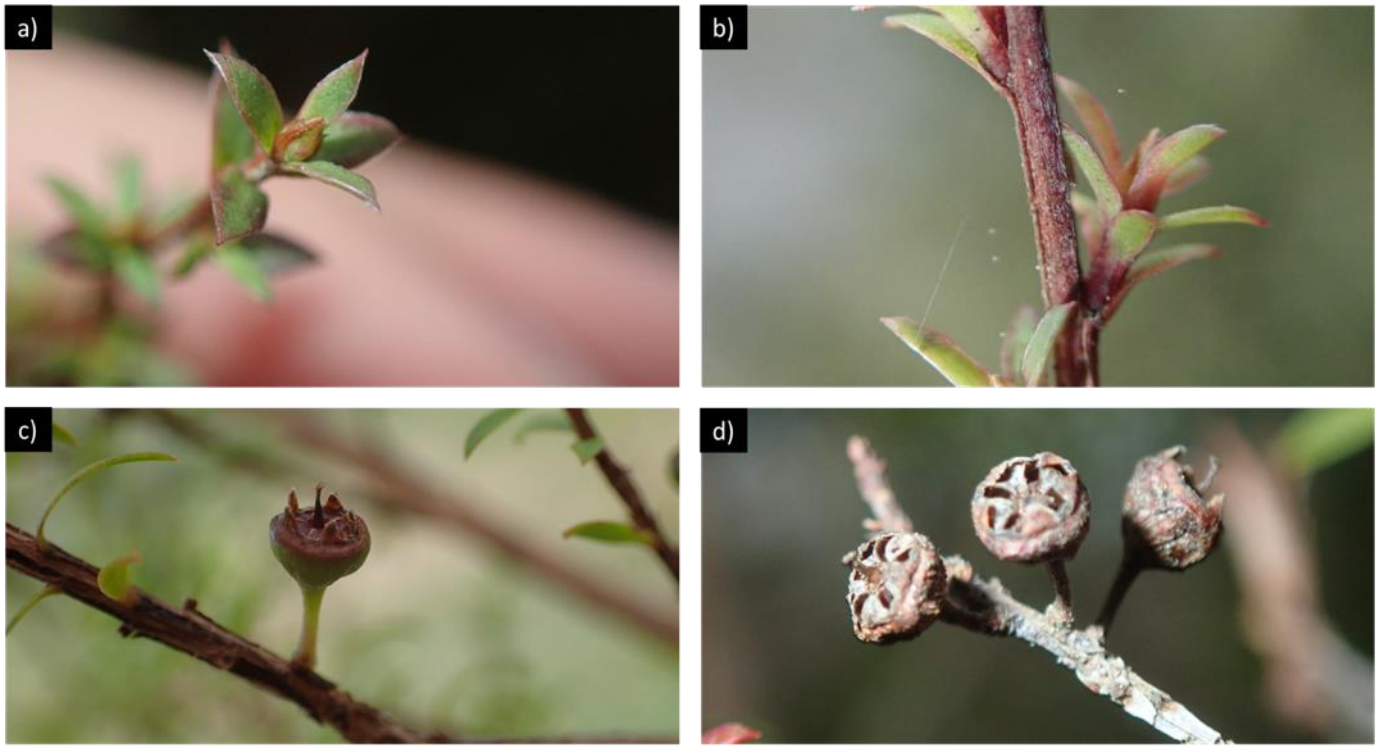


Figure A.4. *Kunzea robusta*

a) leaf bud; b) elongating shoots with soft growth and green stems present;
c) immature fruit; d) mature fruit (opened capsules).

Figure A.5. *Leptospermum scoparium:*
a) main stem measurement; b) leaf bud;
c) elongating shoots with soft growth and green stems present; d) flower bud;
e) flower; f) immature fruits; g) mature fruit (opened capsule).

8.3 Fungicide spray dates

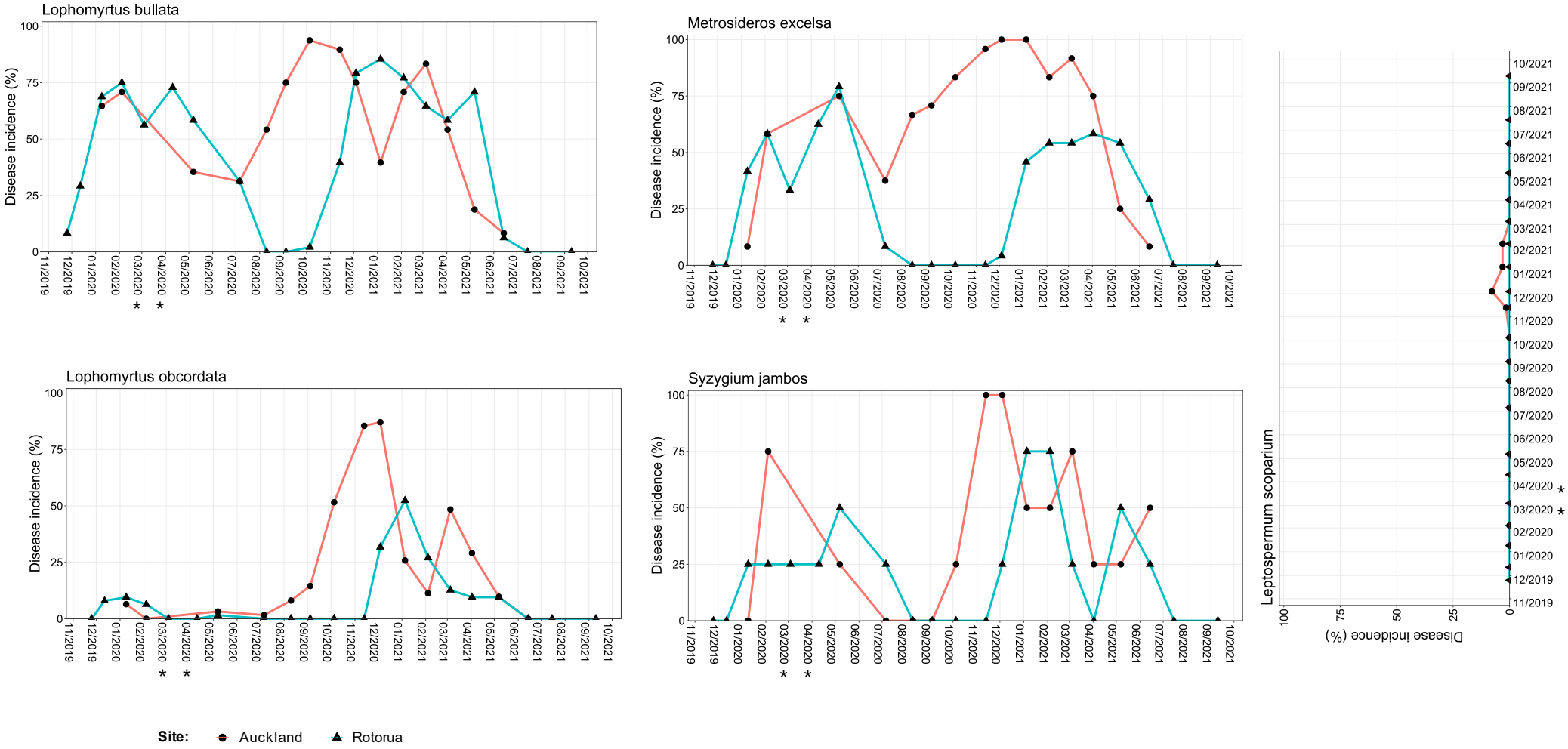
Spray dates for fungicides applied at the Rotorua and Auckland trial sites in the second (2020–21) season.

Fungicide products applied and spray dates for myrtle rust exclusion trials in Rotorua and Auckland.

				Species sprayed			
	Date	Product	Active ingredient	*M. excelsa*	*L. bullata*	*S. jambos*	*L. obcordata*
	Rotorua trial						
1	23/10/2020	Vandia® 250 EC	Triadimenol	+	+	+	
2	27/11/2020	Flint® 500 WG	Trifloxystrobin	+	+	+	
3	12/01/2021	Sercadis®	Fluxapyroxad	+	+	+	
4	26/01/2021	Vandia® 250 EC	Triadimenol	+	+	+	
1	11/02/2021	Flint® 500 WG	Trifloxystrobin	+	+	+	+
2	26/02/2021	Sercadis®	Fluxapyroxad	+	+	+	+
3	26/03/2021	Vandia® 250 EC	Triadimenol	+	+	+	+
4	16/04/2021	Flint® 500 WG	Trifloxystrobin	+	+	+	+
5	14/05/2021	Sercadis®	Fluxapyroxad	+	+	+	+
6	28/05/2021	Vandia® 250 EC	Triadimenol	+	+	+	+
	Auckland trial						
1	6/11/2020	Vandia® 250 EC	Triadimenol	+	+	+	
2	2/12/2020	Flint® 500 WG	Trifloxystrobin	+	+	+	
3	16/012/2020	Sercadis®	Fluxapyroxad	+	+	+	
4	26/01/2021	Vandia® 250 EC	Triadimenol	+	+	+	
5	26/02/2021	Flint® 500 WG	Trifloxystrobin	+	+	+	
6	19/03/2021	Sercadis®	Fluxapyroxad	+	+	+	
7	24/03/2021	Sercadis®	Fluxapyroxad				+
8	17/04/2021	Vandia® 250 EC	Triadimenol	+	+	+	+

8.4 Disease incidence

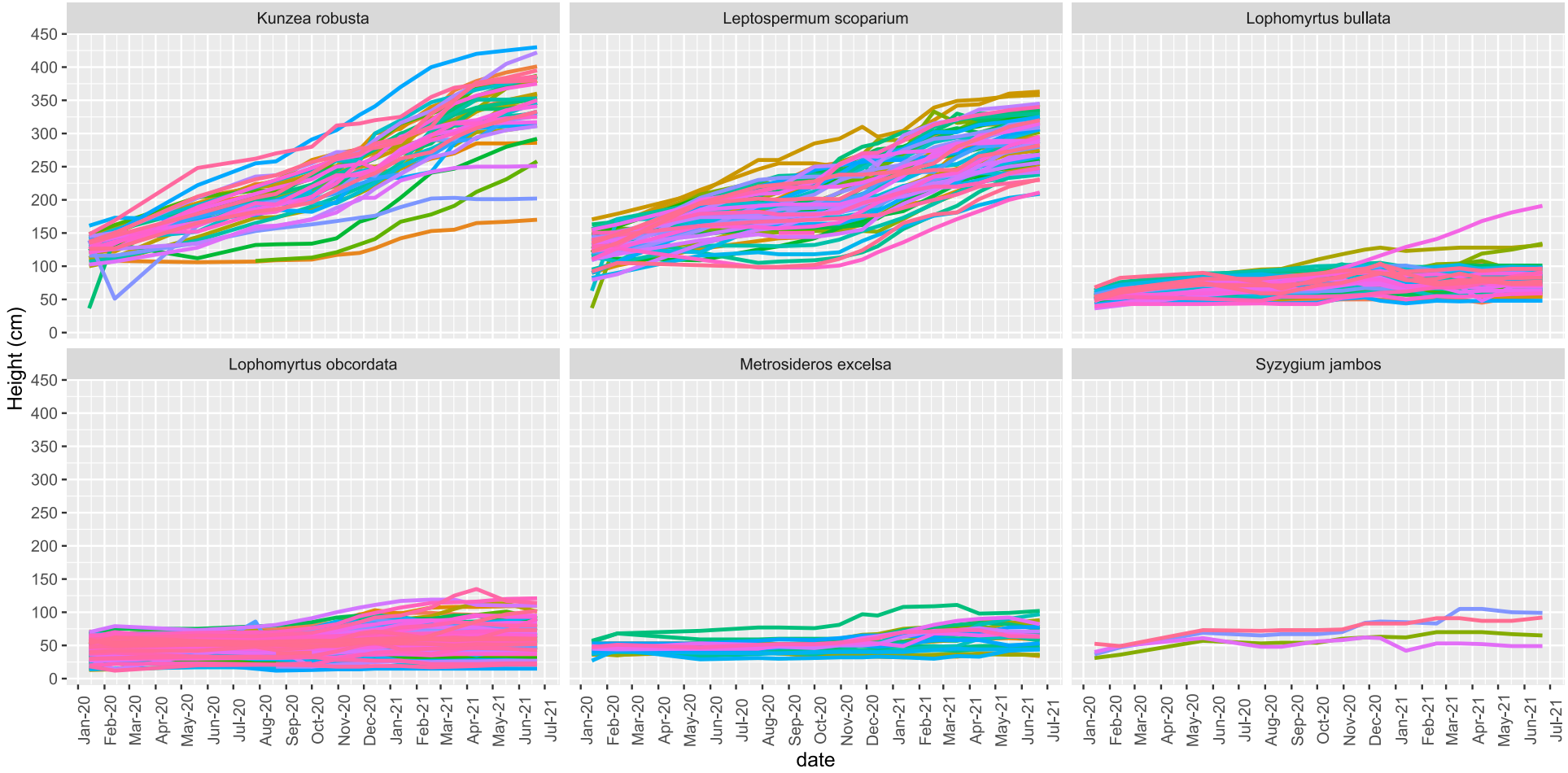
Monthly disease incidence (% of trees with myrtle rust symptoms) at each site for non-fungicide treated trees of the five species that showed myrtle rust symptoms. The asterisks show missing data from Auckland due to COVID-19 lockdown.



8.5 Monthly growth measurements

Monthly growth measurement of individual trees in Auckland (a) and Rotorua (b) trials. Individual trees are represented by single lines.

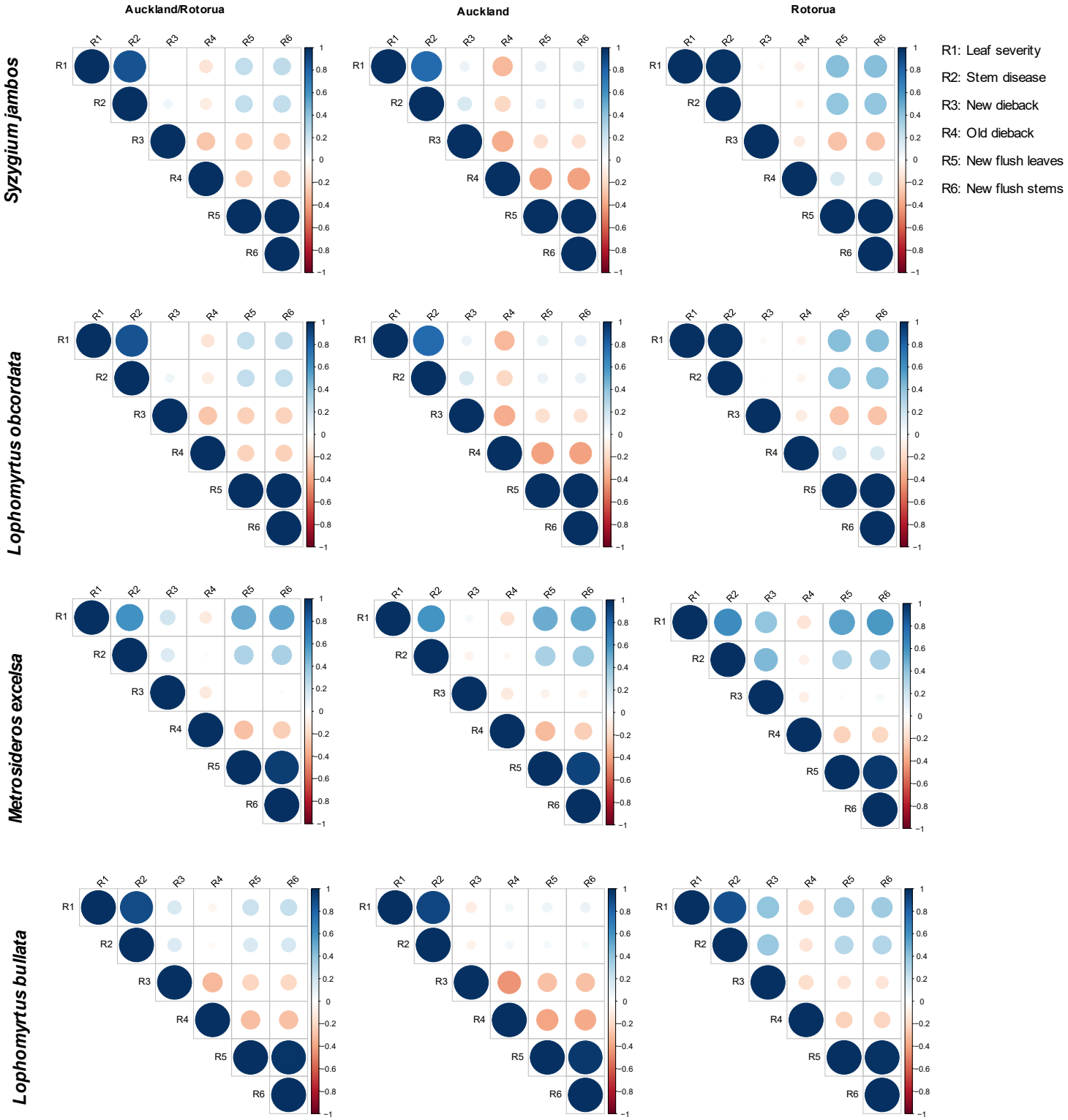
a) Auckland



b) Rotorua


Kunzea robusta
Leptospermum scoparium
Lophomyrtus bullata
Lophomyrtus obcordata
Metrosideros excelsa
Syzygium jambos
Height (cm)
0
50
100
150
200
250
300
Nov-19
Dec-19
Jan-20
Feb-20
Mar-20
Apr-20
May-20
Jun-20
Jul-20
Aug-20
Sep-20
Oct-20
Nov-20
Dec-20
Jan-21
Feb-21
Mar-21
Apr-21
May-21
Jun-21
Jul-21
Aug-21
date

8.6 Pearson correlation matrices between responses variables



8.7 Fungicide effect on *L. bullata* and *M. excelsa* in Rotorua

Analysis of deviance table (Type III Wald Chi square tests) for the three response variables: (1) New leaves, (2) Leaf severity, (3) New dieback using beta generalised linear mixed models for the analysis of *L. bullata* and *M. excelsa* at Rotorua site.

Site	Date of fungicide	Source of variation	% New leaves					% Leaf severity					% New dieback				
			Chi square	df	p z			Chi square	df	p z			Chi square	df	p z		
Rotorua	Nov 2020-Jul 2021	(Intercept)	26.47	1	<0.001	***		127.73	1	<0.001	***		128.41	1	<0.001	***	
		Species	0.77	1	0.38			1.34	1	0.25			0	1	0.96		
		Fungicide	12.49	1	<0.001	***		0.94	1	0.33			0	1	0.98		
		DOA	343.31	8	<0.001	***		206.21	8	<0.001	***		270.97	8	<0.001	***	
		Species * Fungicide	2.3	1	0.13			0.29	1	0.59			0	1	0.98		
		Species * DOA	127.48	8	<0.001	***		59.01	8	<0.001	***		50.43	8	<0.001	***	
		Fungicide * DOA	20.68	8	<0.01	**		34.07	8	<0.001	***		115.42	8	<0.001	***	
		Species * Fungicide * DOA	20.97	8	<0.01	**		14.5	8	0.07			18.53	8	<0.05	*	

df = degrees of freedom of the Chi square statistic.
[z] Significant values are denoted as follows: *p<0.05, **p<0.01, and ***p<0.001.

8.8 Fungicide effect on *L. bullata* and *M. excelsa* in Auckland

Analysis of deviance table (Type III Wald Chi square tests) for the three response variables: (1) New leaves, (2) Leaf severity, (3) New dieback using beta generalised linear mixed models for the analysis of *L. bullata* and *M. excelsa* at Auckland site.

Site	Date of fungicide	Source of variation	% New leaves					% Leaf severity					% New dieback				
			Chi square	df	p^z			Chi square	df	p^z			Chi square	df	p^z		
Auckland	Nov 2020-Jul 2021																
		(Intercept)	7.36	1	<0.01	**		0.29	1	0.587			81.83	1	<0.001	***	
		Species	4.99	1	<0.05	*		5.64	1	<0.05	*		9.35	1	<0.001	***	
		Fungicide	0.61	1	0.434			19.68	1	<0.001	***		4.36	1	<0.05	*	
		DOA	227.87	7	<0.001	***		267.58	7	<0.001	***		596.64	7	<0.001	***	
		Species * Fungicide	0.02	1	0.876			1.56	1	0.212			1.77	1	0.184		
		Species * DOA	130.61	7	<0.001	***		111.53	7	<0.001	***		221.16	7	<0.001	***	
		Fungicide * DOA	72.86	7	<0.001	***		52.02	7	<0.001	***		231.25	7	<0.001	***	
		Species * Fungicide * DOA	36.09	7	<0.001	***		37.92	7	<0.001	***		81.23	7	<0.001	***	

df = degrees of freedom of the Chi square statistic.
^{z} Significant values are denoted as follows: *p<0.05, **p<0.01, and ***p<0.001.

8.9 Fungicide effect on *L. obcordata* in Auckland and Rotorua

Analysis of deviance table (Type III Wald Chi square tests) for the three response variables: (1) New leaves, (2) Leaf severity, (3) New dieback using beta generalised linear mixed models for the analysis of *L. obcordata.*

Site	Date of fungicide	Source of variation	% New leaf				% Leaf severity				% New dieback			
			Chi square	df	p^z		Chi square	df	p^z		Chi square	df	p^z	
Rotorua	Mar-Jul 2021													
		(Intercept)	134.20	1	<0.001	***	330.50	1	<0.001	***	155.51	1	<0.001	***
		Fungicide	0.00	1	0.970		0.77	1	0.380		0.00	1	0.950	
		DOA	4.34	4	0.360		2.02	4	0.730		0.94	4	0.920	
		Fungicide * DOA	7.38	4	0.120		0.55	4	0.970		0.20	4	1.000	
Auckland	Apr-Jul 2021													
		(Intercept)	387.56	1	<0.001	***	244.32	1	<0.001	***	235.68	1	<0.001	***
		Fungicide	0.04	1	0.850		1.61	1	0.200		3.74	1	0.050	
		DOA	2.45	2	0.290		4.66	2	0.100		1.14	2	0.570	
		Fungicide * DOA	2.66	2	0.260		1.51	2	0.470		2.12	2	0.350	

df = degrees of freedom of the Chi square statistic.
^{z} Significant values are denoted as follows: *p<0.05, **p<0.01, and ***p<0.001.

8.10 Fungicide effect on Height growth rate

Results of LMMs testing the effects of fungicide treatment on the (log transformed) seedling Height growth rate (cm/month) for each species and each site.

		L. bullata		*L. obcordata*		*M. excelsa*	
Site	**Variable**	**Estimates**	**p [z]**	**Estimates**	**p [z]**	**Estimates**	**p [z]**
Rotorua	Intercept	0.45	**<0.001*** **	0.62	**<0.001*** **	0.80	**<0.001*** **
	Fungicide (Treated) [a]	0.62	**<0.001*** **	-0.04	0.771	0.15	0.478
Auckland	Intercept	0.86	**<0.001*** **	0.34	**<0.001*** **	0.82	**<0.001*** **
	Fungicide (Treated) [a]	1.05	**<0.001*** **	0.26	**0.029***	0.62	**<0.001*** **

a Dummy variable with a value of 1 when fungicide treated or 0 when fungicide untreated.
[z] Significant values are denoted as follows: *p<0.05, **p<0.01, and ***p<0.001.

8.11 Height growth rate means for fungicide treatments

Means and standard errors (in parentheses) of the Height growth rate (cm/month) at each site and each species.

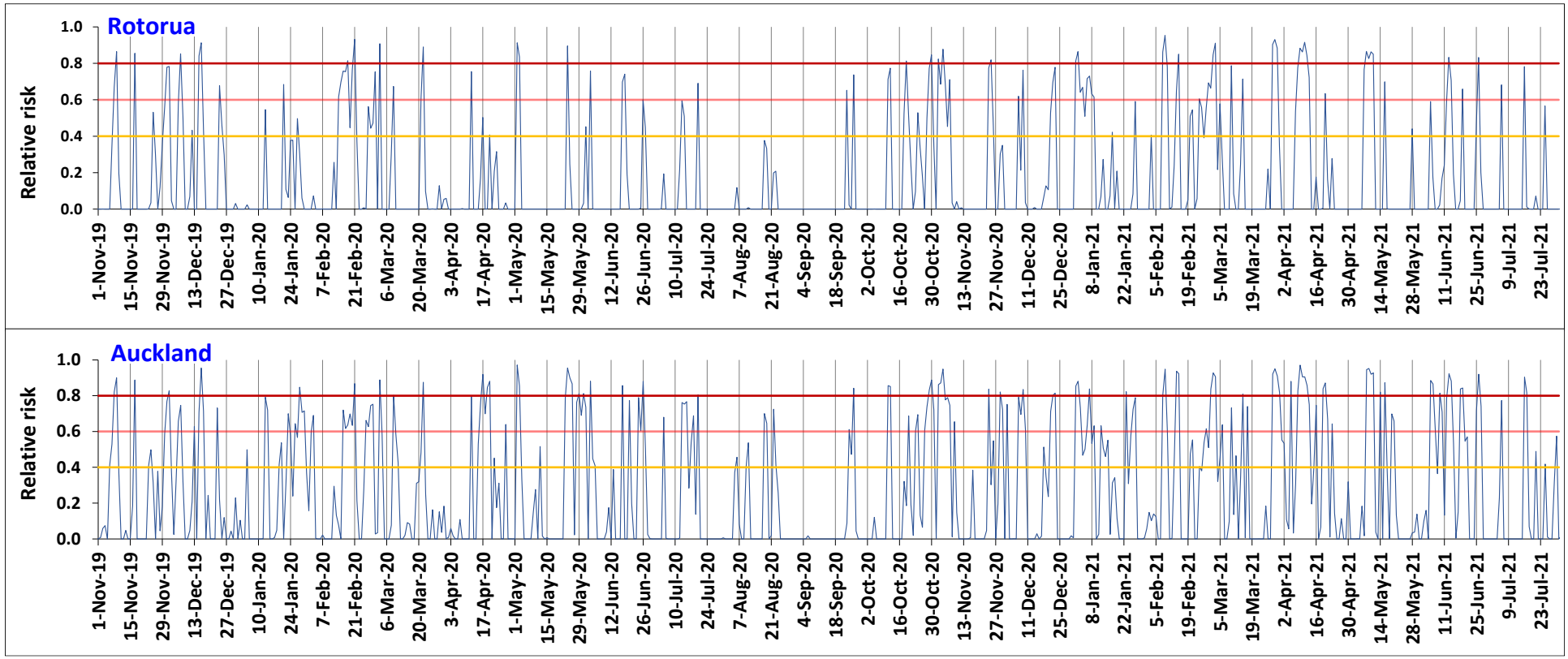
For each species and each site, mean (SE) followed by different upper-case letters indicate statistically significant differences between fungicide treatment (multiple comparison procedures were performed using Tukey contrast at α = 0.05). ns: not significant.

		Fungicide			
Site	Species	Untreated		Treated	
Rotorua	*L. bullata*	0.7	(0.1) A	2.3	(0.3) B
	L. obcordata	1.1	(0.2) ns	1.1	(0.2) ns
	M. excelsa	1.5	(0.4) ns	1.9	(0.4) ns
Auckland					
	L. bullata	2.5	(0.6) A	6.0	(0.3) B
	L. obcordata	0.6	(0.3) A	1.2	(0.2) B
	M. excelsa	1.6	(0.2) A	4.1	(0.7) B

8.12 Predicted infection risk

Daily predicted myrtle rust infection risk at Auckland and Rotorua trial sites between November 2019 and July 2021.

Infection risk was calculated using the Myrtle Rust Process Model (Beresford et al. 2018). Risk category boundaries for moderate (0.4-0.6), high (0.6-0.8) and very high (>0.8) risk are shown.



8.13 Summary of artificial inoculations from Smith pers.com.

		Leaf resistance phenotype	Leaf Susceptible phenotype	Stem resistance phenotype	Stem Susceptible phenotype	Dieback resistance phenotype	Dieback Susceptible phenotype	Note
Species	Family	%L1 or L2	%L3-L5	%S1	%S2-8	%BTD1	%BTD2-5	
Kunzea robusta	1250	27	73	9	91	55	45	
Kunzea robusta	1249	60	40	33	67	73	27	
Kunzea robusta	1248	42	58	8	92	58	42	
Kunzea robusta	1247	42	58	17	83	67	33	
Kunzea robusta	1165	18	82	9	91	18	82	
Leptospermum scoparium	1426	n/a	n/a	n/a	n/a	n/a	n/a	TESTED but results not available
Leptospermum scoparium	1427	n/a	n/a	n/a	n/a	n/a	n/a	TESTED but results not available
Leptospermum scoparium	1428	n/a	n/a	n/a	n/a	n/a	n/a	TESTED but results not available
Leptospermum scoparium	1429	n/a	n/a	n/a	n/a	n/a	n/a	TESTED but results not available
Leptospermum scoparium	1430	n/a	n/a	n/a	n/a	n/a	n/a	TESTED but results not available
Leptospermum scoparium	1431	n/a	n/a	n/a	n/a	n/a	n/a	TESTED but results not available
Leptospermum scoparium	1432	n/a	n/a	n/a	n/a	n/a	n/a	TESTED but results not available
Leptospermum scoparium	1434	n/a	n/a	n/a	n/a	n/a	n/a	TESTED but results not available
Metrosideros excelsa	1176	0	100	n/a	n/a	n/a	n/a	
Metrosideros excelsa	1175	0	100	n/a	n/a	n/a	n/a	
Metrosideros excelsa	1285	0	100	n/a	n/a	n/a	n/a	
Metrosideros excelsa	1284	0	100	n/a	n/a	n/a	n/a	
Metrosideros excelsa	1292	0	100	n/a	n/a	n/a	n/a	
Metrosideros excelsa	1291	5	95	n/a	n/a	n/a	n/a	
Lophomyrtus bullata	unknown	0	100	n/a	n/a	n/a	n/a	
Lophomyrtus bullata	unknown	0	100	n/a	n/a	n/a	n/a	
Lophomyrtus bullata	unknown	0	100	n/a	n/a	n/a	n/a	
Lophomyrtus obcordata	confidential	0	100	n/a	n/a	n/a	n/a	

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