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Monitoring *Austropuccinia psidii* (myrtle rust) on New Zealand Myrtaceae in native forest

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Abstract: *Austropuccinia psidii* (myrtle rust) was first detected on mainland Aotearoa (New Zealand) in 2017 and has established in various urban areas and native forests. To understand the spread of this pathogen and its effect on host species, surveillance for myrtle rust on Myrtaceae in native forests was undertaken in central Te Ika a Māui (North Island). In one site, with confirmed *A. psidii* infection, the rust infected up to 90% of new flush stem and leaves of some ramarama and rōhutu (*Lophomyrtus* spp.), with the pathogen eventually causing dieback of these shoots. The rust also infected developing fruit, causing it to prematurely drop, and infected all seedlings monitored in the site. It is likely that heavily infected trees will die and natural regeneration of *Lophomyrtus* spp. is unlikely; localised extinction is probable. Other Myrtaceae species in the stand, white rātā (*Metrosideros diffusa*) and mānuka (*Leptospermum scoparium*), were also infected but the severity of infection was less on these species than observed on *Lophomyrtus* spp. However, the long-term impact on these species from increasing or sustained disease pressure is unknown. Highly infected plants had decreased insect activity and diversity, highlighting the multi-tropic risk this invasive disease poses. A second site, approximately 15 km from known infected areas, which also contained *Lophomyrtus* spp., remained myrtle rust-free, showing that spread of this disease across landscapes is variable. This is the first monitoring study of myrtle rust on native forests in New Zealand. Continued monitoring is critical to provide information for effective management of this disease.

Keywords: disease progression, *Lophomyrtus*, pandemic biotype

Introduction

Austropuccinia psidii (G. Winter) Beenken is the causal agent of myrtle rust, a highly invasive airborne fungal pathogen that exclusively infects plants belonging to the Myrtaceae family (Glen et al. 2007). *Austropuccinia psidii* is native to South America (Coutinho et al. 1998) but multiple strains of this pathogen have spread worldwide during the last few decades (Stewart et al. 2017) and it is now regarded as a globally invasive pathogen threatening native Myrtaceae, Myrtaceae-related economies, and ecosystems (Pegg et al. 2017). The pandemic biotype (Stewart et al. 2017) of *A. psidii* was first detected on mainland Aotearoa (New Zealand) in May 2017 in Kerikeri (du Plessis et al. 2019; Guy & Barry 2017) but was rapidly found in other regions across Te Ika a Māui (North Island) (Beresford et al. 2018; Toome-Heller et al. 2020).

Myrtle rust symptoms are characterized by lesions with pustules containing masses of bright yellow urediniospores. The pustules form on actively growing new leaves and shoots, as well as on flowers and fruits (Pegg et al. 2014). In the most susceptible host species, myrtle rust lesions can lead to severe defoliation, shoot-tip dieback and plant death (Carnegie et al.

2016). A recent study in Australia reported localised extinction of some host plant species within four years after the pathogen's first detection in a new area, highlighting how devastating myrtle rust can be in natural ecosystems (Carnegie et al. 2016).

There is considerable concern about the long-term impact of myrtle rust in New Zealand, particularly on native species. New Zealand has at least 27 indigenous Myrtaceae taxa (de Lange et al. 2018) that have a significant value in Māori culture (Teulon et al. 2015), New Zealand's economy (Essien et al. 2019), and ecosystems. Assessing the impacts of myrtle rust on susceptible hosts and monitoring disease progress over time under natural conditions will provide crucial information for management strategies against this disease. To date, several native Myrtaceae species are known to be susceptible, including *Lophomyrtus* spp., *Metrosideros* spp. and *Leptospermum scoparium* (mānuka).

The aim of this study was to monitor the occurrence and progression over time of myrtle rust on Myrtaceae in native forests, with a focus on *Lophomyrtus* spp., which is considered to be highly susceptible to *A. psidii*. The genus *Lophomyrtus* comprises two endemic species in New Zealand, *Lophomyrtus bullata* (ramarama) and *Lophomyrtus obcordata*, (rōhutu) (de

Lange et al. 2018). The species naturally hybridize (Rattenbury 1962) and offspring show a range of leaf size and shapes from small heart shaped leaves that are characteristic of *L. obcordata* through to larger bullate leaves of *L. bullata*. Both species and the hybrids grow as shrubs through to small trees in both the Te Ika a Māui (North Island) and Te Wai Pounamu (South Island) (Allan 1961).

Methods

Two sites (A and B), each approximately 500 m in length and 10 m in width, were chosen on the edges of two large remnant forests in the Rotorua district, approximately 13 km apart, where *Lophomyrtus* spp. and other myrtaceous host species were growing as natural populations. The forests were adjacent to lake edges with grassed areas and access tracks. The sites contained *Lophomyrtus* spp., which is considered highly susceptible to *A. psidii*, and plants were selected for monitoring according to accessibility for visual inspection of foliage as monitoring required surveyors to visually inspect leaves to confirm pathogen absence or presence. Permissions to monitor and sample from these sites were granted from local hapū, private landowners, and the Department of Conservation.

In site A five *Lophomyrtus* spp. trees (RA1–5, height range 1.5–8 m) and two *L. scoparium* trees (RA6, 0.5 m; RA7, 2 m) were selected. In site B five *Lophomyrtus* spp. (RO1–5, height range 1–7 m) and one *Metrosideros excelsa* (pōhutukawa; RO6, 4 m) were selected. The *M. excelsa* was not part of the forest and was likely planted as an amenity tree. Within both sites, there were areas where naturally growing *Lophomyrtus* spp. seedlings were abundant under mature trees. In these areas 0.5 × 0.5 m plots of seedlings were established in October 2018 and all seedlings within these quadrats were monitored fortnightly. Two quadrats were established under tree RA2 in site A, with 55 seedlings and two under tree RO5 in site B, with 215 seedlings. Seedlings ranged in height from > 10 cm to 30 cm at the start of monitoring but the age of the seedlings was unknown.

Myrtle rust was first detected in site A in April 2018 on two *Lophomyrtus* trees (RA1 and RA3) (J. Bond, Department of Conservation, pers. comm.) and infected branches from the trees were flagged. From April to October 2018 this site was checked periodically for myrtle rust infection and during this time no new infections on the flagged trees or other trees were observed. In October 2018 regular fortnightly monitoring commenced with data on plant phenology, canopy density, environmental disturbances (including invertebrate presence), and disease symptoms and severity recorded using the New Zealand myrtle rust monitoring form (<https://www.myrtlerust.org.nz/assets/Uploads/Form-MR-surveillance-for-the-website.docx>). All parts of the seedlings and smaller trees were able to be monitored and on these plants all leaves, stems, fruit and flowers were inspected. For larger trees, only a percentage of tree foliage was visible for myrtle rust detection, so the canopy cover was also assessed to determine any changes in density over time. Any invertebrates present on foliage during monitoring activity were photographed. As part of the New Zealand myrtle rust monitoring form, the incidence of myrtle rust on Myrtaceae adjacent (within 3 m) to the selected trees is also recorded. Species of climbing rātā (*Metrosideros fulgans*, *M. diffusa*, and *M. perforata*) were present within the 3 m radius of the individual monitored trees in site A and thus were regularly inspected for symptoms of myrtle rust.

The visual inspection included all young and new foliage, stems, flowers and fruit visible from the ground on the monitored trees, including turning foliage over to inspect the underside. The intensity of myrtle rust sporulation on all plant material (foliage, fruit and flowers) was recorded. For any Myrtaceae plants within 3 m of the monitored trees, all young and new foliage, flowers and fruit were inspected, if rust was found this was noted, but the intensity of rust was not recorded.

Site B is approximately 13 km from site A and 25 km from Rotorua where myrtle rust has established across the city. Site B was checked periodically from April until September 2018 and during this time no myrtle rust was detected. From October 2018 site B was monitored monthly for myrtle rust; had it been detected, fortnightly monitoring would have commenced. Monitoring was undertaken as described for Site A.

Results

At site A, no sign of new myrtle rust infection was seen from April 2018 until 8 November 2018 (7 months) when the first rust symptoms, urediniospores on stems and leaves, were observed on one of the *L. scoparium* (RA6) (Fig. 1g). In late November myrtle rust was detected on the previously infected *Lophomyrtus* sp. RA3, where spores were observed on a small number of new flush leaves and stems. These first symptoms coincided with a marked increase in days with myrtle rust infection risk in this region predicted by the myrtle rust process model (Beresford et al. 2018) (Fig. 2). There were only three infection-risk days from July to September, whereas in October and November there were nine, then 13 in December, which was the peak of infection risk for the season.

In early January 2019, myrtle rust was found on another two *Lophomyrtus* (RA1 and RA2) and by early February 2019, the first symptoms were detected on *Lophomyrtus* RA5. By this time, *A. psidii* had spread across a large number of other *Lophomyrtus* trees in the stand, presumably because of high airborne spore numbers associated with the increasing disease incidence within the stand. The first observation of myrtle rust infecting *Metrosideros* in the site was on 21 March 2019 when spores were found on leaves of *M. diffusa* (Fig. 1f), a Myrtaceae host within a 3 m radius of RA1. Although spores were observed on this plant, no other symptoms have been observed on any of the other *Metrosideros* spp. (*M. perforata* or *M. fulgans*) present within 3 m of the monitored trees. No new spores were observed on infected plants from May 2019 until October 2019. Climatic conditions in New Zealand over the winter months are mostly unfavourable for disease development.

In mid-January 2019, eight weeks after the first symptoms were detected, the disease severity in the three infected *Lophomyrtus* trees (RA1, 2 and 3) was very high with sporulating lesions on 80–100% of new flush leaves and stems (Fig. 1d). This was consistent with the high infection risk over this period predicted by the myrtle rust process model (Figure 2). The first signs of tips dieback were evident in early December and continued to increase; by March 2019 these three infected trees had 50–100% dieback. There was no indication that any infected shoot tips were able to recover from infection. By March, *A. psidii* infection had spread to four of the monitored *Lophomyrtus* spp. trees. In late January and early February when the infection level was high, anecdotal observations of rust presence were made across the stand and



Figure 1 *Austropuccinia psidii* (myrtle rust) infection in monitoring site A. Flowering *Lophomyrtus* sp., December 2018 (a), *Lophomyrtus* sp. infected fruit, January 2019 (b), dry *Lophomyrtus* sp. fruit with old myrtle rust infection, April 2019 (c), infected *Lophomyrtus* sp. shoots, January 2019 (d), bronze beetle *Eucolaspis brunnea* feeding on infected *Lophomyrtus* sp. leaves (e), infection on *Metrosideros diffusa*, March 2019 (f), infection on *Leptospermum scoparium*, October 2018 (g), dieback on a *Lophomyrtus* seedling from myrtle rust infection, February 2019 (h).

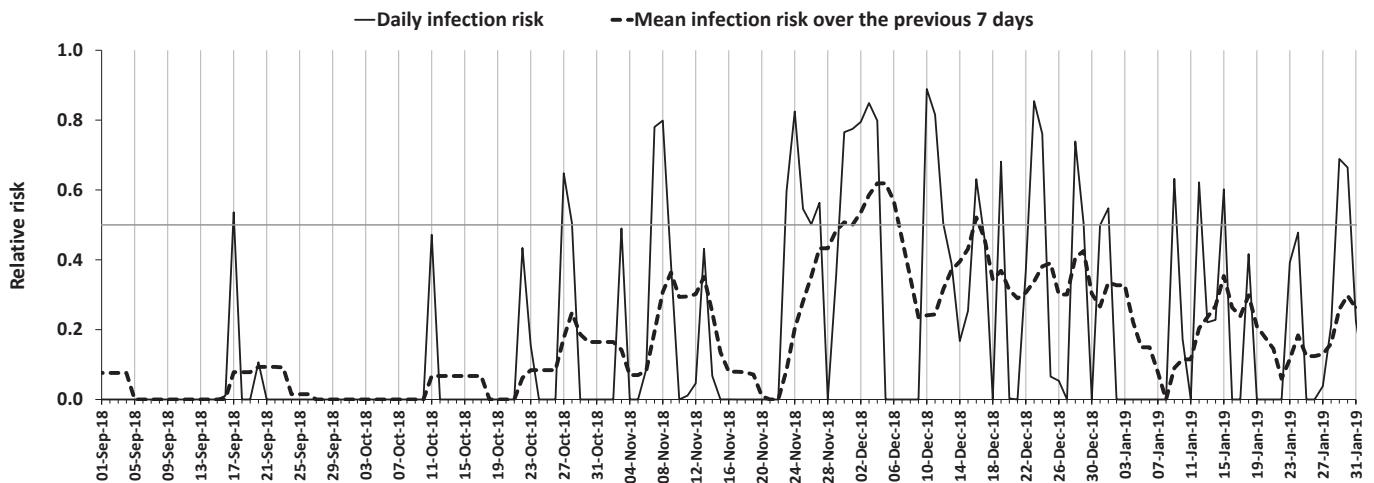


Figure 2. Risk of myrtle rust infection at Rotorua Airport from September 2018 to January 2019. Predictions were made by the myrtle rust process model using temperature, relative humidity and solar radiation (Beresford et al. 2018). Substantial infection is expected on days with risk > 0.5, if the pathogen is present. Weather data for Rotorua Airport were supplied by the Meteorological Service of New Zealand Limited.

infection was found to extend to many (more than 100) other *Lophomyrtus* spp. from the forest edge up to approximately 20 m into the forest. However, not all trees in the stand were infected, other parts, including the region where RA4 was located, have remained myrtle rust-free.

Flower buds emerged on *Lophomyrtus* in late November with full flowering in mid-December. No myrtle rust spores were detected on any flower buds or flowers (Fig. 1a). Developing fruits were present in early January and became infected over January/February (Fig. 1b). On 10 January 2019, 80–100% of the fruit present on RA3 was infected. Infected

fruits were opened in the field and spores were discovered on the inside of the fruit and present on the seeds within; whether the spores were infecting the embryos was unknown. By mid-February 2019 most of the infected fruit had dried up (Fig. 1c) and prematurely dropped to the ground.

Myrtle rust was not present on any of the seedlings in the 0.25 m² seedling quadrates from November through to late December 2018, but by 10 January 2019 76% of the seedlings had myrtle rust symptoms, and by 7 February 2019 all the live seedlings had myrtle rust symptoms (Figures 1h and 3). Although the onset of seedling infection did not directly

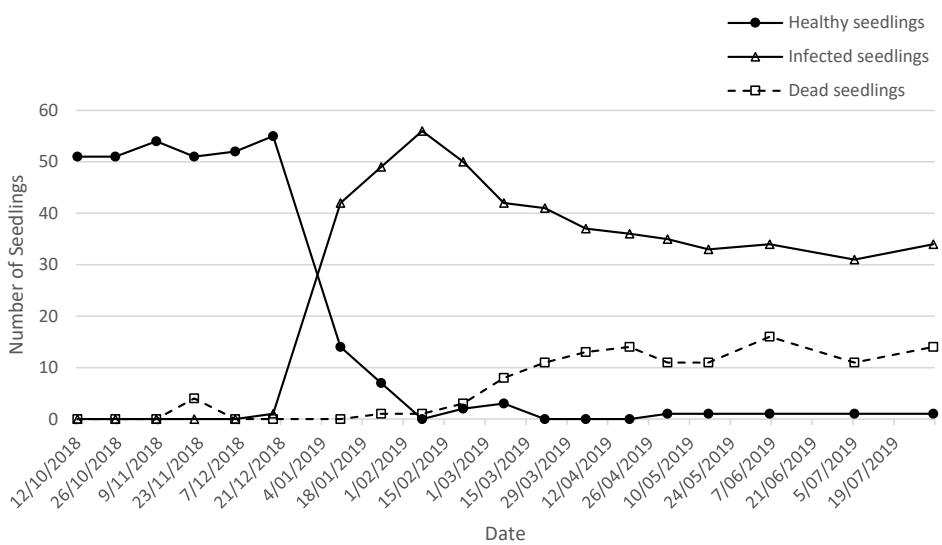


Figure 3. Infection of *Lophomyrtus* spp. hybrid seedlings by *Austropuccinia psidii* (myrtle rust) from November 2018 to August 2019. Seedlings were assessed as healthy with no symptoms of myrtle rust, infected with symptoms of myrtle rust (yellow urediniospores) or dead. Seedling death from myrtle rust was not distinguishable from natural attrition.

correlate with the start of the high predicted infection risk period (Fig. 2), it is likely that the lag was due to inoculum build up on the infected RA2 tree. Seedling death due to myrtle rust started to occur in February and 20% of the monitored seedlings (11 seedlings) had died by the end of March 2019 (Fig. 3). In May 2019, thirty-three seedlings were still alive, and some had produced new flush which became infected immediately. Some new flush was uninfected which may allow those seedlings to survive until next season.

In Site A anecdotal observations of the invertebrates on infected *Lophomyrtus* spp. were made throughout the monitoring period, with photographs taken of invertebrates observed. Thirty invertebrates (all different taxa) were photographed in January and early Feb, this included species from the orders Coleoptera, Lepidoptera (Geometridae, Psychidae), Phasmatodea, Araneae, Hemiptera, and Hymenoptera. Some of the invertebrates on infected shoots were able to be identified to species or genus, this included: bronze beetles (*Eucolaspis* sp.; Fig. 1e), native bees (*Leioproctus* sp.; *Colletidae*), crab spiders (*Sidymella* sp.; Thomisidae), and dew drop spiders (*Argyrodes antipodianus*; Theridiidae). From mid-February to the end of March the only invertebrates observed on infected *Lophomyrtus* spp. were Araneae and some minute larvae that appeared to be scavenging on infected plant material (these were not able to be identified from photos) that had not been observed previously.

In site B, no myrtle rust was detected in the five *Lophomyrtus* spp. (RO1–5), the one *Metrosideros excelsa* (RO6) or in the two 0.25 m² quadrats of *Lophomyrtus* hybrid seedlings, at October 2019 this site was still myrtle rust-free. The purpose for monitoring this site was to determine how rapidly the disease could spread across the district. Despite the close proximity of site B to site A (13 km) and to Rotorua (25km), both having confirmed myrtle rust infection, and presence of susceptible hosts in site B, symptoms of myrtle rust have not been detected.

Discussion

This study is the first report of the impact of myrtle rust on Myrtaceae in native ecosystems in New Zealand and shows that *Lophomyrtus* spp. are highly susceptible and could face

localised extinction, similar to that observed in Australia (Carnegie et al. 2016). Evidence of the potential for localised extinction includes our observations that all fruit became infected and prematurely dropped and the high degree of seedling death over a short period of time. Artificial inoculation of *Lophomyrtus* spp. with *A. psidii* has also shown the species is highly susceptible and thus far, there is no indication of any resistance in this species (Smith et al. 2020). This highlights the need to focus on conservation and germplasm storage of *Lophomyrtus* spp. and other at-risk species before myrtle rust becomes further established in native ecosystems, making it impossible to collect seed for severely affected species. In Australia the first reports of localised extinctions occurred within four years of the pathogen establishing (Carnegie et al. 2016). If localised extinction of *Lophomyrtus* spp. were to occur in New Zealand, this indicates the window of opportunity to conserve these species in areas already infected is limited.

Infection levels on other native Myrtaceae in the site were low. Nothing is known about the genetic resistance of *L. scoparium* or *Metrosideros* spp. in the two sites monitored, although artificial testing of *L. scoparium* against *A. psidii* has shown this species is susceptible and there are multiple forms of resistance in the population (Smith et al. 2020). During the incursion response there was a high number of myrtle rust detections on *Metrosideros* spp., but the majority of these detections were on *Metrosideros excelsa* and hybrids, and not on species of climbing rātā (Beresford et al. 2019; Toome-Heller et al. 2020). If these species are tolerant or resistant to myrtle rust, it will be interesting to see if they are able to withstand repeated infection by the rust. Continued disease pressure as inoculum levels increase could see these species further impacted.

Although *A. psidii* can spread across large distances and can spread quickly within hosts or adjacent trees, this study shows spread across stands may not always progress as quickly as we had assumed, potentially providing time for disease management procedures to be implemented in newly infected stands. Consistent with this, landscape level spread to site B had not occurred 18 months after myrtle rust had been first detected at site A and in Rotorua, showing the importance of minimising human-mediated spread where possible to uninhabited areas. The lack of spread was not thought to be because the plants were more tolerant or resistant, but rather the

pathogen had not been transferred to these areas. Interestingly, this same pattern of sporadic landscape spread of myrtle rust was noted during the first years post incursion in north-eastern Australia (G. Pegg, Queensland Government Department of Agriculture and Fisheries, pers. comm). As levels of inoculum rise, it is expected that the pathogen will continue to spread to uninfected trees and areas that have climatic environments conducive to myrtle rust.

It is unknown what invertebrate species may be detrimentally affected by the potential loss of Myrtaceae species and what invertebrates or molluscs may subsequently change their diet to feed on myrtle rust spores (Ramsell & Paul 1990). There is limited information on invertebrate communities associated with *Lophomyrtus* spp. and similarly the ecosystem role, diversity and abundance of *Lophomyrtus* spp. are also poorly understood, making the effects of this disease difficult to quantify. Considering the importance of invertebrates on bird survival and pollination, knowledge to underpin an ecosystem-level conservation plan is critical. The ecosystem consequences of myrtle rust impacts extends beyond plant loss. The effect of myrtle rust on invertebrate communities highlights the risk this disease poses to multiple trophic levels in forests across New Zealand where Myrtaceae are present.

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