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Symptom development and latent period of *Austropuccinia psidii* (myrtle rust) in relation to host species, temperature, and ontogenic resistance

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Abstract

Myrtle rust (*Austropuccinia psidii*) is an invasive species causing damage to Myrtaceae species in natural and managed ecosystems in many countries. To better understand myrtle rust epidemiology we studied latent period (LP) and ontogenic resistance in relation to temperature on three susceptible hosts (*Metrosideros excelsa*, *Lophomyrtus bullata* × *L. obcordata* and *Syzygium jambos*). The latent period curve was U-shaped, with latent development >0 from between 8 and 10 °C, depending on the host, to 32 °C. Optimum range was 22–28 °C with minimum LP of 5–7 days. Peak spore production occurred over about 2 weeks, starting about 1 week after the LP ended. Some spore production continued for 1–2 months. Comparison of the LP data with field temperatures indicated that the uredinial stage of *A. psidii* can overwinter in the latent phase in temperate areas of New Zealand and southern Australia and, therefore, uredinial or telial reinfection is not required during winter. The LP information was used to correct the LP function in a New Zealand myrtle rust climatic risk model. The transition of emergent leaf and stem tissues in susceptible Myrtaceae genotypes from susceptible to immune (ontogenic resistance) was characterized in terms of uredinium density and LP. Onset of ontogenic resistance was closely linked to the degree of leaf expansion, with fully expanded leaves being immune to infection. Because ontogenic resistance restricts infection to periods when growth flushes occur, understanding it is crucial for explaining the seasonality of myrtle rust development in the natural environment.

KEYWORDS

age-related resistance, generation time, infection cycle, primary inoculum, rust symptoms, urediniospore inoculation

1 | INTRODUCTION

Myrtle rust (*Austropuccinia psidii*) is an invasive plant pathogen that infects and produces symptoms on about 480 Myrtaceae species worldwide (Soewarto *et al.*, 2019), representing about 14% of all Myrtaceae species, and the host range is considered to be expanding (Carnegie and Lidbetter, 2012; Morin *et al.*, 2012; Pegg *et al.*, 2014b). On highly susceptible species, uredinial epidemics cause death of leaves, stems, flowers, and fruit, and, in severe cases, plant death (Glen *et al.*, 2007). It has been implicated in localized species

extinction in some natural habitats in Australia (Carnegie *et al*., 2016; Pegg *et al*., 2017) and is a cause for concern in New Zealand, where it arrived in 2017 (Beresford *et al*., 2018).

Nine biotypes of *A. psidii* have been identified through molecular analysis, two of which occur outside the area of origin in Central and South America. These are the South African biotype, found only in South Africa, and the widespread pandemic biotype, which has been identified in Hawaii, Australia, New Zealand, Colombia, Southeast Asia, China, and Japan (Granados *et al*., 2017; Stewart *et al*., 2018; du Plessis *et al*., 2019). Information on the host range of each of the biotypes and the degree of host susceptibility is currently incomplete.

Since establishing in New Zealand, myrtle rust has been found on at least 12 native and several exotic species of Myrtaceae. While all Myrtaceae species were initially considered to be at risk, incursion surveillance data from the Ministry for Primary Industries (MPI) suggested that the native species *Metrosideros excelsa* (*pōhutukawa*), *Lophomyrtus bullata* (*ramarama*), *Lophomyrtus obcordata* (*rōhutu*), and natural hybrids between the latter two species were particularly vulnerable. Some other key species, such as *Leptospermum scoparium* (*mānuka*), which is important to the honey industry, appeared to be less at risk (Beresford *et al*., 2019). Climatic modelling showed that myrtle rust infection risk was greatest in the northern North Island, decreasing southward and at higher altitude, but with significant risk also in northern and western areas of the South Island (Beresford *et al*., 2018).

Many aspects of myrtle rust epidemiology and disease management are currently poorly understood, but an improved understanding can be achieved through a quantitative epidemiology approach, such as that employed for cereal rusts since the 1960s that led to more effective disease management and resistance breeding (Rapilly, 1979; Johnson, 1980). A parameter that has not been characterized for myrtle rust, but which is crucial in determining epidemic rate, is the latent period from infection to production of new urediniospores (Teng and Close, 1978). Knowledge of the *A. psidii* latent period would help to interpret pathogen detection data during incursion surveillance, to explain the seasonal dynamics of myrtle rust development, and to develop disease management strategies. Rust latent periods are generally shorter on more susceptible hosts (Johnson, 1980) and shorter at higher temperature (Beresford and Royle, 1988; Hernandez Nopsa and Pfender, 2014). The latent period for rust fungi, unlike the infection process, is not dependent on external wetness or high relative humidity (Roelfs *et al*., 1992). Accurate *A. psidii* latent period information is also needed to calibrate the Myrtle Rust Process Model that was developed for climatic risk prediction when *A. psidii* first arrived in New Zealand (Beresford *et al*., 2018). The parameters for the latent period submodel were estimated from limited published information available at the time, and definitive data for different hosts over a range of temperatures are required to verify the accuracy of the latent period function.

An epidemiologically important feature of *A. psidii* development on susceptible Myrtaceae hosts is the restriction of infection to young, expanding host tissues. Leaves and stems that are initially susceptible to infection when they first emerge become resistant as they expand and mature (Tommerup *et al*., 2003; Glen *et al*., 2007). In biotrophic pathogens, this ontogenic or developmental resistance is reported to be associated with maturation of juvenile plant tissues and is not a result of prior challenge by a pathogen or the presence of other internal or external microbes (Whalen, 2005; Develey-Rivière and Galiana, 2007). Development of ontogenic resistance to *A. psidii* in the tissues of Myrtaceae species has not yet been characterized quantitatively.

The aims of this study were to quantify the response of the *A. psidii* latent period to temperature, characterize symptom development, and characterize development of ontogenic resistance in the susceptible New Zealand native species *M. excelsa*, *L. bullata*, and *L. obcordata*. In addition, *Syzygium jambos* (rose apple), which is native to Southeast Asia, was included for comparison because of its high susceptibility to myrtle rust (Tessmann *et al*., 2001), its worldwide distribution (Morton, 1987), and its widespread use in *A. psidii* research as a model host plant and for multiplying inoculum (Ruiz *et al*., 1989).

When this study was initiated, *A. psidii* was under official control by the MPI in New Zealand and it was not permissible to propagate the pathogen in the laboratory or field. Initial experiments were therefore undertaken in a controlled environment facility at the Biosciences Precinct in Brisbane, Australia (Figure 1). Subsequently, permission was obtained from MPI and the Environmental Protection Authority to carry out field inoculations in Auckland, New Zealand using dry transfer of spores from infected to healthy plants within areas where *A. psidii* was present. The study was therefore done both in Brisbane and in the field in Auckland using field collections of the pandemic biotype of *A. psidii* (Pegg *et al*., 2014b; du Plessis *et al*., 2019) collected in each country.

2 | MATERIALS AND METHODS

2.1 | Plant material

For experiments in Brisbane, clonal plant material of *M. excelsa* 'Mini New Zealand Christmas Tree' and a local selection of *S. jambos* were

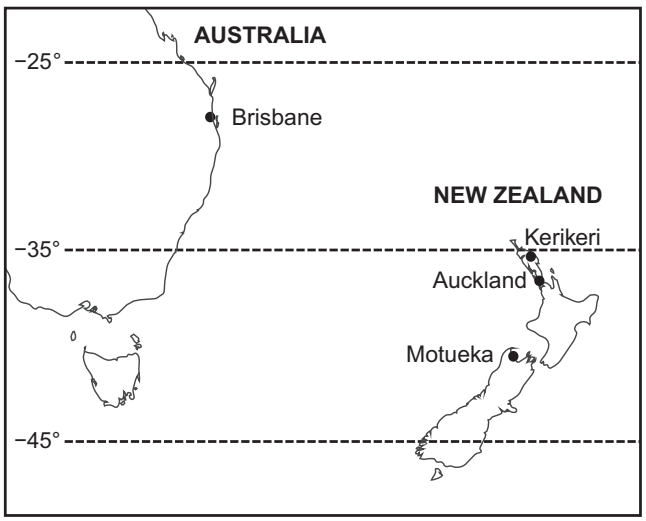

FIGURE 1 Locations in New Zealand and Australia where myrtle rust studies were conducted including lines of south latitude

used. For the Auckland experiments, clonal material of a *Lophomyrtus* sp. selection 'Red Dragon', a natural hybrid between *L. bullata* and *L. obcordata*, an unnamed clonal selection of *M. excelsa*, and seedlings of *S. jambos* that had been prescreened for high susceptibility to *A. psidii* were used.

2.2 | Controlled environment experiments

A series of inoculation experiments with controlled temperatures was performed in Brisbane using *M. excelsa* and *S. jambos*. Four temperature treatments were provided for each experiment by three temperature-controlled cabinets and a roof-top shade house, where temperature fluctuated naturally (mean 18–25 °C; range 10–31 °C). Actual temperatures experienced by the plants for the duration of each experiment were recorded using portable temperature and relative humidity loggers (EasyLog, EL-USG-2, Lascar Electronics). For each temperature treatment and species, 10 replicate plants were spray-inoculated with a water suspension of 10^5 *A. psidii* urediniospores/ml, plus 100 µl/ml of Tween 20. The spores had previously been collected, dried, and stored at −80 °C. Infection was established with a 24 hr wet period under a plastic tent in the dark at 18 °C, using conditions similar to those described by Ruiz *et al.* (1989). After the wet period, plants were incubated at nominated temperatures (indicated in the results section) and assessed daily for symptom development on the top five leaf node positions and stem internodes (position 1 = the youngest visible leaf pair). Uredinium density on infected tissue was recorded in four categories: 0 = zero; 1 = 1–10; 2 = 11–100; 3 = 101–500 uredinia/cm^2. Mean uredinium densities were calculated using the midpoints of these categories (0, 5, 50, and 250).

2.3 | Field experiments

Field inoculation experiments in Auckland used *M. excelsa*, *Lophomyrtus* sp. 'Red Dragon', and *S. jambos*. Each inoculation was made on five shoots per plant on four glasshouse-raised potted plants (20 shoots per inoculation). Inoculations in Auckland used dry transfer of *A. psidii* urediniospores from field-infected leaves using an artist's paintbrush to the top six or seven leaf pairs of the shoots to be inoculated. Dry transfer of spores was used in Auckland because biosecurity restrictions prevented spraying of spore suspensions. There was no evidence that the inoculation method affected the latent period results. This method produced maximum uredinium densities of 10–200/cm^2, which was similar to those from spore suspension inoculation with 10^5 spores/ml. Natural day/night temperature fluctuations in different seasons were used to obtain a range of different mean temperatures. The seasonal average temperatures during the study were: summer 20.6 °C, autumn 16.3 °C, and winter 11.5 °C). During autumn and winter, elevated temperatures (mean 19–20 °C) were obtained for some experiments by placing plants into a plant growth tent (Homebox HL40; 40 × 40 × 120 cm; https://www.homebox.net/en/kontakt/) with a 45 W LED light panel and a heating pad). Lower night-time temperatures (4–5 °C) during warmer winter weather were obtained by placing plants into a portable 12 V cooler (Waeco CFX-65W). Temperature loggers (as described above) accompanied the plants during each experiment.

2.4 | Symptom development at low temperature

Symptom development on *Lophomyrtus* sp., from first symptoms at the end of the incubation period to the cessation of sporulation at the end of the infectious period, was studied in relation to mean temperature in the field in Auckland during winter using natural and modified temperatures, as described above. Actively growing shoots (15–20) on two replicate plants per treatment were inoculated, then misted with water and covered with plastic bags to generate a wet period of 24 hr at 15–20 °C. After the wet period, plants were incubated under four different temperature regimes: (1) tent (19–20 °C; control); (2) field (natural temperature); (3) cooler overnight (4–5 °C); and (4) cooler overnight until first symptoms, then tent. Regime 4 investigated whether an extended period of low temperature during the presymptomatic period resulted in mortality of uredinial infections. Symptom development was recorded according to six stages: 1 = first symptoms; 2 = first uredinium erupted; 3 = 100% of uredinia erupted; 4 = peak sporulation begins; 5 = decline in sporulation begins; 6 = <10% of uredinia still sporulating. Exponential functions were fitted to the data to illustrate trends in the response of symptom stage to temperature using the Trendline function in Microsoft Excel.

2.5 | Symptom development in relation to leaf node position

Uredinium density and latent period were studied on actively growing shoots of *M. excelsa* to quantify the degree to which leaves at different positions on a shoot were susceptible or resistant to infection. Data for three temperatures (18 °C fluctuating, 20 °C constant, and 28 °C constant) from one of the inoculation experiments in Brisbane were used. There were 10 replicate plants (shoots) for each temperature treatment. Six leaf node positions per shoot were assigned numbers (1 = top) 7 days after inoculation, at about the time rust symptoms first appeared. Percentage leaf expansion at that time, relative to the area of a fully expanded leaf, was typically as follows: position 1, 0%–5%; position 2, 5%–15%; position 3, 25%–50%; position 4, 50%–75%; position 5, 75%–100%; and position 6, 90%–100%. The rate of leaf area expansion was greatest at positions 2 and 3. At the time of inoculation, leaves at position 1 had emerged on only a few shoots. Unemerged leaves did not become infected and leaves that had partly emerged at inoculation time became infected only at the leaf tip. Statistical separation of means for uredinium density and latent period with

respect to leaf position was done by analysis of variance (ANOVA) in GenStat 17th Edition (VSN International) using the Bonferroni adjustment ($\alpha = 0.05$).

2.6 | Latent period in relation to temperature

The response of latent period in the three host species to mean temperature during the latent period was determined using data from Brisbane and Auckland. Latent period was defined as the number of days from inoculation to the first erupted uredinium (stage 2) on ≥50% of the leaf pairs across the replicate shoots. The response was modelled as the latent development rate (1/latent period), using the following function, which is a modification of a function used for wheat stem rust (Hernandez Nopsa and Pfender, 2014):

$$1/\text{latent period} = C \times A \times (\text{Temp} - T_{\min}) \times \left\{1 - e^{[B \times (\text{Temp} - T_{\max})]}\right\}, \quad (1)$$

where C is 1 for hourly or 24 for daily temperature data; A is the linear rate of increase; Temp is the mean temperature during the latent period; T_{min} is the minimum temperature for latent development; T_{max} is the maximum temperature for latent development; and B is the exponential rate of decrease.

Latent period, therefore, = 1/latent development rate.

Parameters A, B, T_{min} and T_{max} were fitted to the latent period and temperature data using the nonlinear regression facility in Minitab 18.1 (Minitab, Inc.).

2.7 | Modelling seasonal latent period

The interaction of the latent period response for *Lophomyrtus* sp. (Equation 1, using the appropriate parameter values from Table 1) with seasonal field temperatures during 2019, as an example, was modelled for three locations where myrtle rust occurs (Figure 1), representing different climate types: (a) subtropical, Brisbane Airport (Queensland), Australia (lat. −27.39°, long. 153.13°); (b) warm temperate, Kerikeri (Northland), New Zealand (lat. −35.21°, long. 173.97°); and (c) cool temperate Motueka (Tasman), New Zealand (lat. −41.15°, long. 173.01°). Both the New Zealand sites were at Plant & Food Research field stations. The instantaneous daily value of the latent period was calculated from daily mean air temperature, using a 5-day moving average to smooth day-to-day fluctuations, and plotted over 12 months (January–December 2019). If the temperature is $<T_{min}$ or $>T_{max}$, the latent period value is infinitely large and under those circumstances values were truncated to 100 days. To investigate how long *A. psidii* can remain latent at low winter temperatures, the progress of hypothetical latent infections that started on selected days during the year was simulated as the cumulative proportion of the latent period completed each day. A value of 1 indicated the end of the latent period. This was done for Motueka and Kerikeri in 2018 and 2019. Brisbane was not included because the warm temperatures there produced very little seasonal variation in latent period.

3 | RESULTS

3.1 | Symptom development

The development of myrtle rust symptoms following inoculation of *M. excelsa* and *S. jambos* (Brisbane and Auckland) and *Lophomyrtus* sp. (Auckland only) showed that uredinia developed only on the upper leaves of shoots. For *M. excelsa*, uredinia developed only on the top four or five inoculated leaf pairs and on the petioles of the youngest, rapidly expanding leaves. The leaves present on the potted *M. excelsa* plants were of the juvenile leaf form, lacking hairs on the undersurface and having a soft texture. The first sign of infection on the youngest leaves (leaf pairs 1–3) was distortion of leaf shape, whereas first symptoms on older leaves (leaf pairs 4 and 5) were bumps or

TABLE 1 Parameter estimates and standard errors (*SE*) for four parameters fitted to *Austropuccinia psidii* latent development rate (1/latent period) data in relation to mean air temperature during the latent period for three Myrtaceae species

Host species	*df*	*A*	T_{min} (°C)	T_{max} (°C)	*B*
Metrosideros excelsa		0.00058	10.6	32.4	0.1780
SE	4	0.00043	3.31	1.2	0.2424
Lophomyrtus sp.		0.00048	7.6	32.1	0.2848
SE	10	0.00003	0.48	0.2	0.0482
Syzygium jambos		0.00065	8.5	32.2	0.2501
SE	7	0.00031	1.7	0.6	0.2439
MRPM prelim.		0.00030	1.0	30.0	0.1000
MRPM corrected		0.00048	7.6	32.1	0.2848

Note: See Equation 1 for the fitted function. Also shown are the preliminary parameter values for the MRPM (Beresford *et al.*, 2018) and the corrected values derived from *Lophomyrtus* sp. in this study.

Abbreviations: *A*, linear rate of increase; *B*, exponential rate of decrease; MRPM, Myrtle Rust Process Model; T_{min}, minimum temperature for latent development; T_{max}, maximum temperature for latent development.

blisters, purple or necrotic spots, and unerupted uredinia, followed by a few erupted uredinia. A previous inoculation experiment (data not presented) compared the juvenile and adult leaf forms of *M. excelsa* 'Vibrance' and showed no difference in the timing or severity of symptom development between the two forms. On *Lophomyrtus* sp. the top two leaf pairs at the time of inoculation developed uredinia, where the first was just emerging and the second was partly expanded. Petioles of these leaves, the top internode and the emerging shoots in the axils of the second leaf pair also developed uredinia. Occasionally, uredinial lesions developed on the lamina of the third leaf pair. The first sign of infection on emerging *Lophomyrtus* sp. leaves was shape distortion. On the laminae of older leaves, red spots or blotches appeared, particularly at low temperature (<10 °C). On petioles and internodes, unerupted uredinia were the first sign of infection. On *S. jambos*, uredinia developed on leaves, petioles, and on the stem at the leaf node on the top two to three leaf pairs. The first sign of leaf infection on expanding leaves was shape distortion followed by blisters and red blotches. On the fourth leaf pair, necrotic spots and sparse uredinia occasionally developed.

3.2 | Symptom development at low temperature

The fitted lines in Figure 2 show that symptom development in *Lophomyrtus* sp. was slower at low mean temperatures, with first symptoms (stage 1) appearing, on average, 26 days post-inoculation at 10 °C, down to 5.8 days at 20 °C. The infectious period from first uredinium erupted (stage 2) to the end of sporulation (stage 6) lasted for 64 days at 10 °C, down to 43 days at 20 °C and the period of peak spore production (stages 4–5) was 17 days at 10 °C and 14 days at 20 °C. In temperature regime 4, the low mean temperature of 9.7 °C during the symptomless period, including

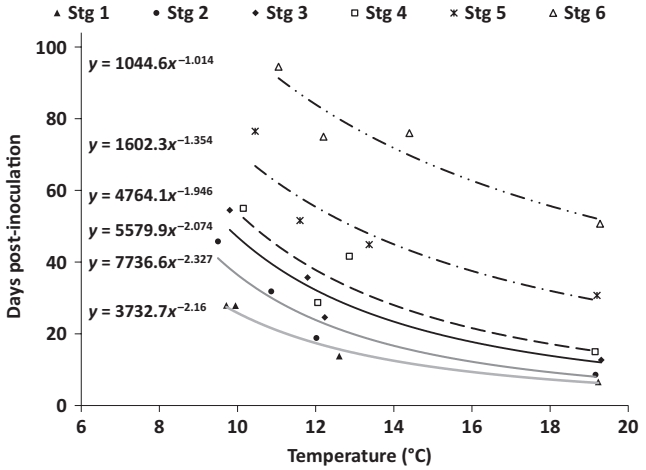

FIGURE 2 Myrtle rust symptom development on *Lophomyrtus* sp. in relation to temperature, as the number of days from inoculation to appearance of six symptom stages: 1, first symptoms; 2, first uredinium erupted; 3, 100% of uredinia erupted; 4, peak sporulation begins; 5, decline in sporulation begins; 6, <10% of uredinia still sporulating. Exponential functions are fitted to illustrate trends in the data

4–5 °C at night, had no effect in reducing subsequent uredinium density on leaves and stems when the temperature was raised, based on visual comparison between the four regimes. The extended period under field conditions in regimes 2 and 3 resulted in visibly lower spore production compared with the protected tent environment in regimes 1 and 4, and was caused by weathering of the uredinial lesions by wind and rain.

3.3 | Symptom development in relation to leaf node position

Uredinium density on inoculated *M. excelsa* leaves was greatest at positions 2 and 3 at 18 and 20 °C (Figure 3a). Density was lower on the more mature leaves at positions 4 and 5, with very few uredinia at position 5. At 28 °C, density was greatest at positions 3 and 4 and very low at position 5. No uredinia developed at position 6 at any temperature (not shown in Figure 3), although necrotic spotting was sometimes observed at position 6. Most leaves at position 1 and some at position 2 had not emerged at the time of inoculation. These escaped infection and were excluded when calculating the means in Figure 3. Low uredinium densities occurred on the newly emerged leaves at positions 1 and 2, not because they were resistant, but because the very small leaf area at inoculation limited the amount of infection and resulted in few uredinia on the expanded leaves. The apparent shift in resistance at 28 °C to one leaf pair further down the stem, compared with 18 and 20 °C, was an artefact of the leaf numbering, where the higher leaf emergence rate at 28 °C allowed some shoots to produce an extra leaf node by the time the nodes were numbered.

Latent period was shortest at 28 °C on the youngest leaves (position 2) and became significantly longer with increasing leaf maturity (Figure 3b). This trend occurred to a lesser extent at 20 °C, but not at 18 °C, where the latent period was constant in relation to leaf maturity at the time of inoculation. The greater resistance of the more mature leaves at positions 4 and 5 was closely related to the degree of leaf expansion. Leaves that were >75% expanded at inoculation tended to be highly resistance or immune. Susceptibility was greatest in the most rapidly expanding leaves at positions 2 and 3.

The trend for decreasing uredinium density with increasing leaf node position was also observed in the latent period study (below) of *Lophomyrtus* sp. and *S. jambos*. For these species, leaves at positions 3 or 4 were the lowest on which uredinia developed and no uredinia were observed at position 5, although on *S. jambos* necrotic spots were sometimes observed at position 5. Susceptibility to infection on all three hosts ended at about the time leaves were 90%–100% expanded, irrespective of incubation temperature.

3.4 | Latent period in relation to temperature

Myrtle rust latent period was strongly related to mean air temperature. The data points from both the constant temperature experiments in Brisbane and the fluctuating temperatures in the Brisbane shade house and field experiments in Auckland all lay along the same fitted

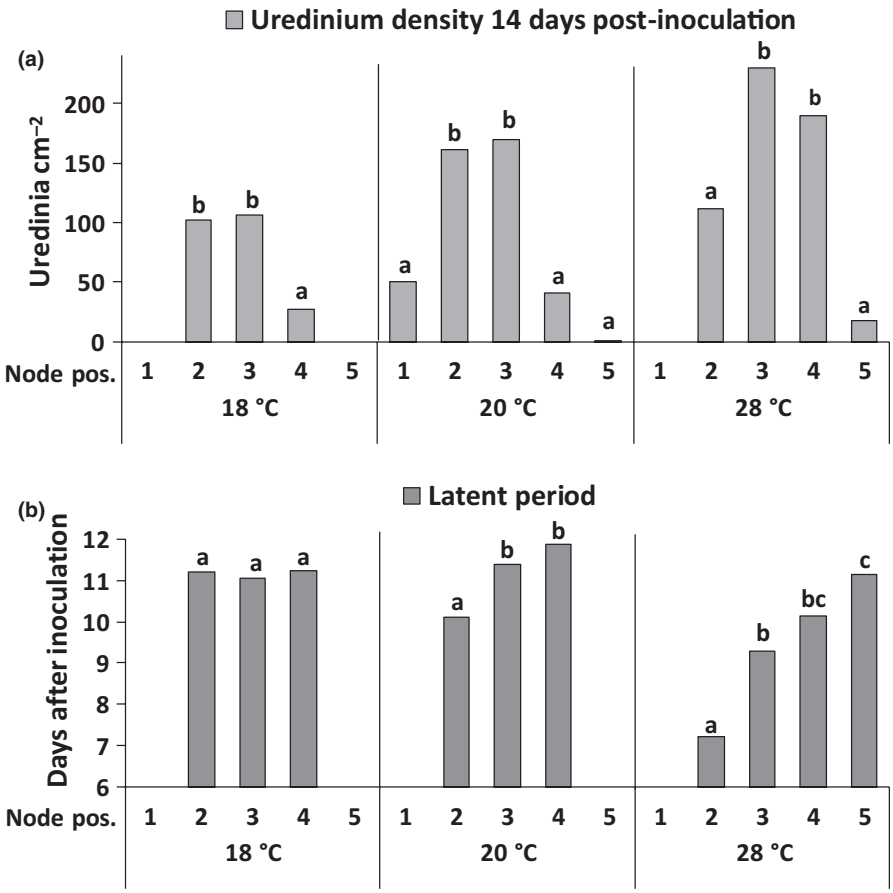

FIGURE 3 Mean uredinium density (a) and latent period (b) on *Metrosideros excelsa* leaves infected by *Austropuccinia psidii* for the top five leaf node positions on stems (position 1 = top). Three experiments were conducted at three different mean temperatures: 18, 20, and 28 °C. Bars accompanied by the same letter within each temperature are not significantly different ($p < .05$; Bonferroni test). The overall temperature effect was significant for uredinium density ($p < .001$), but not for latent period ($p = .344$). The temperature × leaf position interaction was significant for both uredinium density and latent period ($p < .001$)

response curves (Figure 4a–c). Latent period response was relatively flat near the optimum temperature of 25 °C, between about 18 and 28 °C, but increased markedly either side of that temperature range. The fitted responses were similar for all three species, with the shortest latent periods (4.5–6.7 days) closer to the maximum than the minimum temperature. The fitted value of the T_{min} parameter for latent development was higher for *M. excelsa* (10.6 °C) than for *S. jambos* (8.8 °C) or *Lophomyrtus* sp. (7.6 °C), although the high standard error for the *M. excelsa* value indicates that the estimate is rather imprecise (Table 1). The minimum latent period was shorter for *S. jambos* (4.5 days) than for *Lophomyrtus* sp. (5.7 days) or *M. excelsa* (6.7 days).

The preliminary latent period response to temperature used in the latent period submodel of the Myrtle Rust Process Model (Figure 4d; Table 1) predicted latent development continuing down to 1 °C, which the new results show substantially overestimates the low temperature development rate of *A. psidii*. It also predicted a maximum temperature of 30 °C, which underestimated the development rate at high temperature. In addition, the preliminary response predicted a minimum latent period of 11.6 days, which is nearly twice as long the values observed in this study.

For both *M. excelsa* and *Lophomyrtus* sp., the increase in latent period at mean temperatures >30 °C was associated with an adverse effect on the health of host leaves and uredinial development became variable, with lower uredinium densities and necrosis of infected tissues. For *M. excelsa* at very low temperature, latent development appeared to be arrested, but infections were not killed by the low temperature. For example, for one inoculation in the Brisbane controlled temperature facility that was held initially at a constant 8 °C, no symptoms developed for one month, but when the temperature was raised to 12 °C, symptoms appeared, although uredinia were slow to develop at that temperature. This is consistent with the low temperature study of *Lophomyrtus* sp. in Auckland described above, where latent development was able to resume following overnight temperatures as low as 4 °C.

3.5 | Modelling seasonal latent period

During the summer months (January, February, and December, 2019), the instantaneous daily values of predicted latent period on *Lophomyrtus* sp. were remarkably similar for Brisbane, Kerikeri and Motueka (Figure 5) and were close to the predicted minimum for that host of 5.7 days. By contrast, from late autumn (April) to spring (October), values differed markedly between locations, with frequent, extremely long values (truncated to 100 days) at Motueka during winter (June–August) when temperatures were $<T_{min}$ (7.6 °C). Predicted latent period was also long at Kerikeri during winter, peaking at 50 days, although the temperature there was always $>T_{min}$, the lowest value being 9 °C. At Brisbane, the lowest winter temperature was 15 °C and predicted latent period increased only slightly during the winter months, up to a maximum of 12 days. Although only a single year of modelled latent period data are shown, the pronounced

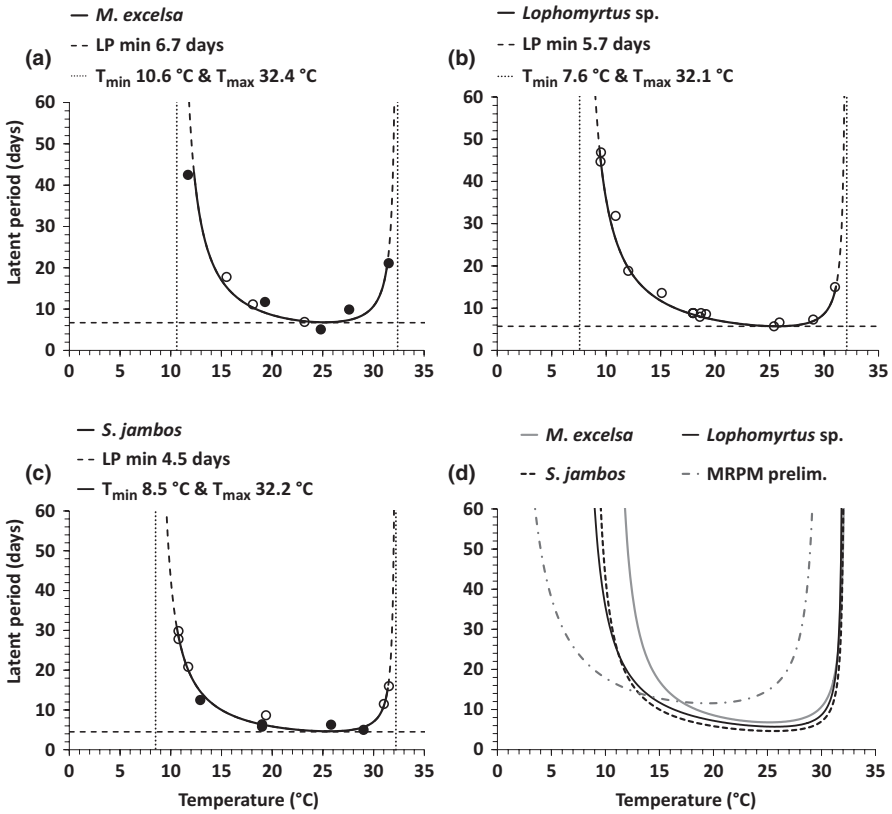

FIGURE 4 Modelled relationships between *Austropuccinia psidii* latent period and temperature for *Metrosideros excelsa* (a), *Lophomyrtus* sp. (b), and *Syzygium jambos* (c). Closed symbols are constant temperature data and open symbols are fluctuating shade house or field data. Dashed lines indicate extrapolation of the fitted curves beyond the experimental data points. See Equation 1 for the fitted function and Table 1 for the parameter estimates. (d) Compares latent period responses for each species with the preliminary estimate used in the Myrtle Rust Process Model (MRPM; Beresford *et al*., 2018)

difference in seasonal patterns between the three locations highlights their geoclimatic differences and these would be affected to only a small degree by year-to-year differences in temperature patterns.

Modelled latent period duration for hypothetical infections starting at different times during 2018 and 2019 showed, for Motueka, maximum latent periods of 118 days in 2018 and 115 days in 2019 for infections starting in May (late autumn) and ending in September (early spring; Figure 6). At Kerikeri, in 2018, the maximum modelled latent period was only 24 days for infections starting and ending in July and, in 2019, only 20 days for infections starting ending and in August. At Motueka in both 2018 and 2019, there was a convergence of the modelled latent period end dates in spring, such that the end of latency for infections initiated any time between May and September occurred within one month of each other during September or early October. This convergence was caused by springtime warming that shifted temperatures upwards into the part of the response curve where a small increase in temperature causes a substantial decrease in latent period (Figure 4). The convergence happened a little earlier in 2018 than 2019, reflecting slightly different annual temperature patterns. No such convergence occurred in the slightly warmer climate at Kerikeri, where mean daily temperatures were never below T_{min} and where all simulated infections ended within a month of starting.

4 | DISCUSSION

This study is the first to characterize the response of the *A. psidii* uredinial latent period to temperature on susceptible hosts. Our results show that latent development is favoured by relatively warm temperatures, with a U-shaped response of latent period that decreases with temperature from between about 8 and 10 °C, depending on the host, to an optimum range between 22 and 28 °C and a maximum of 32 °C. The minimum latent period over the optimum range is 5–7 days, which is shorter than the 12 days previously reported in the literature (Glen *et al*., 2007).

The differences between the three species used in the latent period–temperature response curve study showed that *M. excelsa* had the highest T_{min} value (10.6 °C) and the longest minimum latent period (6.7 days). Although most of the *M. excelsa* data points came from the Brisbane experiments, three came from the field in Auckland (open circles in Figure 4). These lay along the same response line as the Brisbane controlled environment data, suggesting that the different curve for *M. excelsa* was a species difference, rather than due to where or how the experiments were conducted. Similarly for *S. jambos*, data points from both Brisbane and Auckland were part of the same temperature response. *Lophomyrtus* sp. had the lowest T_{min} value (7.6 °C), indicating that latent development can occur at a slightly lower temperature in this host. T_{max} values were about 32 °C for all three species. The latent period information from this study has been used to modify the latent period submodel of the Myrtle Rust Process Model, using the response for *Lophomyrtus* sp.

The study of symptom development at low temperature in *Lophomyrtus* sp. showed that, in Auckland, the infectious period of *A. psidii* continued for 1–2 months after the end of the latent period, but the period of peak spore production lasted only about 2 weeks,

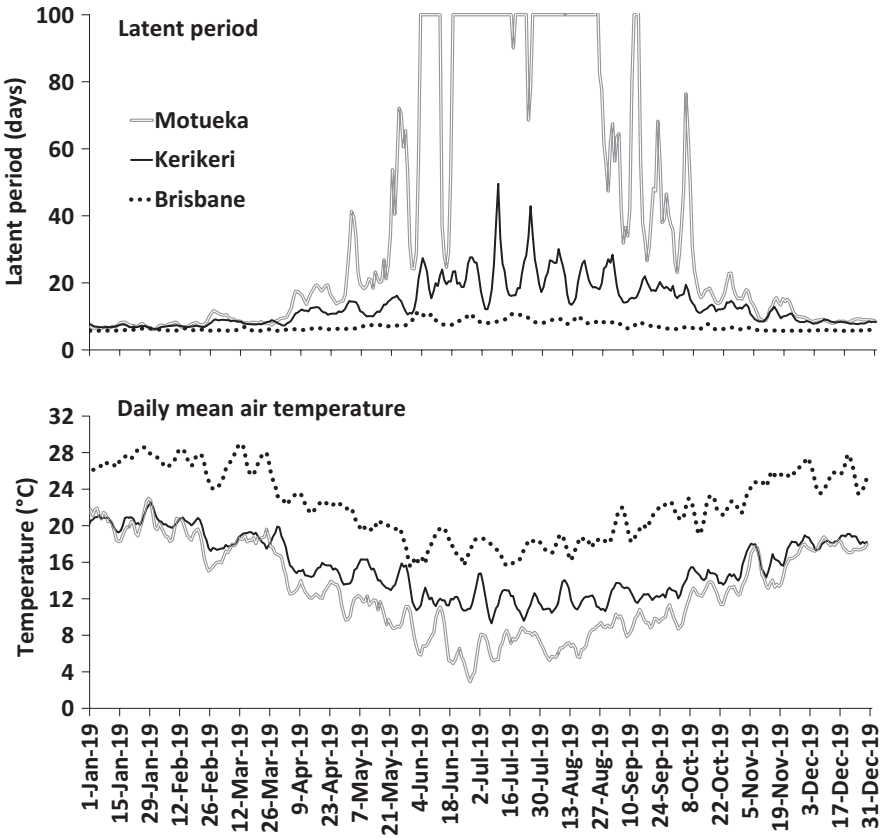

FIGURE 5 Daily latent period values of *Austropuccinia psidii* on *Lophomyrtus* sp. modelled using a 5-day moving average of daily mean air temperature for Brisbane (Queensland), Australia; Kerikeri (Northland), New Zealand; and Motueka (Tasman), New Zealand. Modelled latent period values are truncated to a maximum of 100 days when the temperature is below the minimum for latent development (7.6 °C)

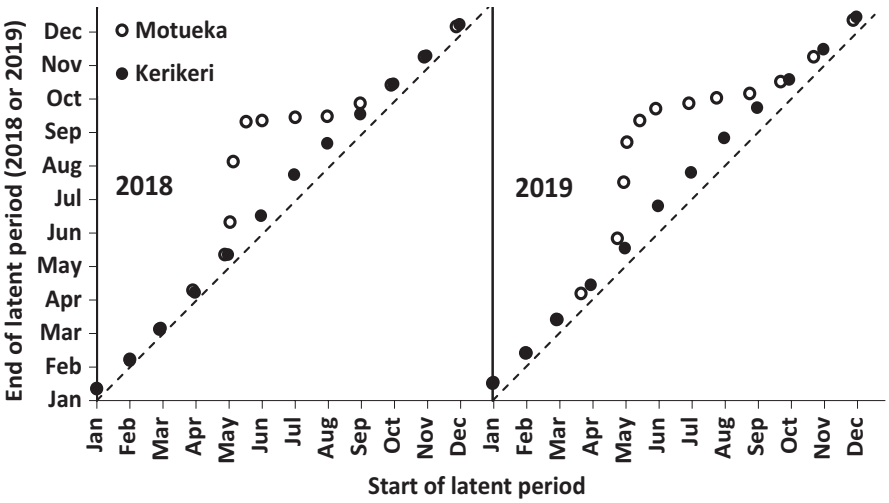

FIGURE 6 Predicted end dates for the latent period of *Austropuccinia psidii* on *Lophomyrtus* sp. for simulated infections starting on various dates in 2018 and 2019 at Motueka (lat. −41.15°, long. 173.01°) and Kerikeri (lat. −35.21°, long. 173.97°) in New Zealand. The vertical distance from the dashed lines to each data point is the duration of the latent period, which varied from 6 to 24 days at Kerikeri and 7 to 118 days at Motueka

beginning approximately 1 week after the first uredinium erupted. Although latent development was static or very slow at mean temperatures below about 10 °C, development resumed again when the temperature was raised to 20 °C. Overnight temperatures as low as 4 °C did not cause mortality of uredinial infections. Reversible low temperature inhibition of latent development was also observed in *M. excelsa* in one of the Brisbane inoculations that was initially held at a constant 8 °C for 1 month. It is not known whether latent infection within host tissues could survive temperatures below 0 °C. For a frost-tender species, like *M. excelsa*, freezing temperatures could kill tissues and thereby eliminate latent infection, but for more frost-hardy hosts, like the *Lophomyrtus* sp., it remains to be determined whether *A. psidii* mycelium could survive periods of subzero temperatures.

The low temperature study provided the first evidence that the uredinial stage of *A. psidii* is capable of overwintering in the latent phase in cool temperate climates. This means that reinfection during winter via either the uredinial stage or the telial stage (McTaggart *et al.*, 2018) is not necessary for perennation between seasons. Further insight into uredinial overwintering came from modelling seasonal

latent period in *Lophomyrtus* sp. This suggested that infections starting in late autumn could remain latent for 4 months under Motueka winter temperatures and that the latent period end dates for simulated infections initiated any time from late autumn to early spring converged in spring when temperatures started to rise. This indicates that, in cool climates, *A. psidii* primary inoculum from infections the previous season becomes available over a relatively short period in spring. This phenomenon would also be expected in other susceptible Myrtaceae host species, and indeed in other rust pathosystems, depending on how the latent period response curve interacts with seasonal temperature.

Seasonal temperature also affects host growth and because infection by *A. psidii* is driven by new growth flush, occurrence of myrtle rust epidemics in a region must also depend on how the host's growth physiology is adapted to local temperature. For example, a warm-adapted host species might not be greatly affected by myrtle rust in a cooler climate if the growth rate, and consequently the amount of susceptible tissue, is always limited by low temperature. Another influence on seasonal host growth is amount and seasonal pattern of rainfall, and in unmanaged plant ecosystems this may be more important for myrtle rust development than temperature. In managed systems, like plant nurseries, water availability is generally maintained to ensure high plant vigour, which will also cause rust susceptibility to remain high.

New Zealand and the southern states of Australia (southern New South Wales, Victoria, South Australia, and Tasmania) have warm to cool temperate climates, unlike the tropical and subtropical areas where *A. psidii* originates. Mean daily temperatures in the North Island and northern South Island of New Zealand, where myrtle rust risk is greatest (Beresford *et al.*, 2018), vary between about 7 °C in winter and 23 °C in summer. Temperatures are therefore always suboptimal for *A. psidii* latent development. In contrast, mean daily temperatures in subtropical Queensland (Brisbane) range from about 13 °C in winter to 30 °C in summer and are therefore generally more optimal for myrtle rust development. However, in tropical climates with extended periods with mean temperatures above 32 °C, latent development is likely to be inhibited and this raises the question of how *A. psidii* oversummers in high temperature areas. Significant myrtle rust damage has been reported from the tropics, both in wet areas, for example north Queensland (Pegg *et al.*, 2014b), and in dry areas, for example Darwin and Melville Island in the Northern Territories (Westaway, 2016), and the disease also occurs close to the equator in Singapore (du Plessis *et al.*, 2019). In tropical ecosystems, local climatic and seasonal effects could provide at least a few cooler months allowing *A. psidii* infection and latent development to occur, with temperatures still high enough to promote rapid disease development. The possible role of teliospores in high temperature survival of *A. psidii* is worthy of investigation. The telial stage does not appear to be involved in overwintering for *A. psidii*, as is the case for many rust species (e.g., Desprez-Loustau *et al.*, 1998; Hacquard *et al.*, 2013), and teliospores were not observed during the overwintering studies in New Zealand. Determining if teliospores play an ecological role at high temperatures would help to understand when the greatest likelihood of genetic recombination within *A. psidii* occurs (McTaggart *et al.*, 2018).

Climate change from global warming is likely to increase the risk of myrtle rust in temperate climates because the period in each year when both *A. psidii* latent development and host growth are active will increase. In the tropics, climate warming may induce longer periods above the maximum temperature for latent development and may therefore reduce the risk of myrtle rust by inhibiting the infection cycle.

Ontogenic resistance restricts infection to actively growing shoots and therefore confines natural epidemics to periods when growth flushes occur, with important implications for seasonal disease development (Tessmann *et al.*, 2001). This study quantified ontogenic resistance in terms of decreasing uredinium density and increasing latent period for successive leaf pairs produced on *M. excelsa* stems. This transition was also observed, but not quantified, in *Lophomyrtus* sp. and *S. jambos*; the lowest leaf node position at which uredinia could develop varied slightly between the three host species.

Ontogenic resistance has been described for many biotrophic and hemibiotrophic pathogens, including mycosphaerella leaf blotch on eucalypts (Ganapathi, 1979), *Phytophthora parasitica* on tobacco (Hugot *et al.*, 1999), *Venturia inaequalis* on apple (Li and Xu, 2002), *Uncinula necator* on grapevine (Ficke *et al.*, 2003), and *Podosphaera aphanis* on strawberry (Asalf *et al.*, 2014). The pathogenicity traits that have been used to study ontogenic resistance include colony growth, disease incidence, disease severity, and sporulation. In this study, we examined symptom expression, uredinium density, and latent period, which are widely used for the different purpose of characterizing phenotypic expression of plant resistance genes to rust infection. Host necrosis, indicating a hypersensitive reaction, and uredinium density are used in host resistance rating scales for *A. psidii* (Junghans *et al.*, 2003; Pegg *et al.*, 2014a) and latent period is a component of partial resistance to cereal rusts known as "slow rusting" (Johnson, 1980).

In contrast to plant genetic resistance, mechanisms of ontogenic resistance are generally poorly understood and, for *A. psidii*, few studies have been made. Because *A. psidii* infects by direct penetration through the cuticle, rather than through the stomata, the development of a cuticular barrier seems a likely mechanism for ontogenic resistance development. Xavier *et al.* (2015) found that prepenetration inhibition of *A. psidii* on *Eucalyptus grandis* was associated with increasing thickness of cuticular wax on older leaves. However, unfortunately, that study only examined fully expanded leaves of increasing age and was therefore not relevant to the period when ontogenic resistance develops, as shown in this study. The symptoms that appear on leaves during ontogenic resistance development are, as noted above for the three Myrtaceae genera in this study, blotches and necrotic spots that are consistent with a hypersensitive reaction. This indicates penetration has already occurred at the time resistance develops and suggests a postpenetration resistance mechanism, rather than a prepenetration barrier from cuticular wax development.

Attempts to understand ontogenic resistance mechanisms in other pathosystems have used microscopy to study infection and colonization processes (Ficke *et al.*, 2003) and possible links to changes in phenolic and sugar composition (Calonnec *et al.*, 2018). However, the only general conclusion appears to be that ontogenic resistance is closely linked to leaf age or physiological maturity and that it is a very consistent and predictable characteristic for a given pathosystem. Our observation that the period over which ontogenic resistance develops is closely associated with the period of leaf and internode expansion suggests that the control of both tissue expansion and ontogenic resistance may be linked in some way. Tissue expansion in plants is controlled by cell elongation mediated through the phytohormone auxin, although the exact mechanism by which auxin controls both tissue expansion and apical dominance is not fully understood (Majda and Roberts, 2018). Auxin has also been linked to plant defence responses (Kazan and Manners, 2009) and the possibility that auxin-mediated effects may influence the onset of ontogenic resistance in Myrtaceae to *A. psidii* is worthy of further investigation.

This study has increased our understanding of myrtle rust epidemiology in relation to symptom development, latency, and host ontogenic resistance. Interpreting how these factors interact leads us to propose that, in cool temperate climates, the annual disease cycle of myrtle rust exhibits a pronounced epidemic season. The season starts when a small amount of carryover primary inoculum appears in spring and infects new flush shoots on susceptible hosts. This initiates infection cycles that become increasingly rapid as temperatures warm, producing increasing amounts of secondary inoculum that lead to severe disease damage to new emerging shoots. Assuming moisture conditions remain suitable for infection and new shoot growth continues, disease severity peaks between summer and early autumn. As temperatures decrease during autumn, both pathogen and host growth rates slow and infection frequency decreases. In warmer temperate climates, a low rate of sporulation, host growth, and reinfection may continue throughout winter. In cooler temperate climates, the pathogen enters a latent overwintering phase that can continue for several months, ending as temperatures rise again in spring. Our study provides important new quantitative information about the biology of myrtle rust and its interaction with climate that will facilitate new research to better understand the epidemic dynamics of this important invasive species in both natural and managed plant ecosystems.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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