MPI 18608 Project Report

Topic 1.2 — Identification of asymptomatic periods

Prepared for Ministry for Primary Industries
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Contents

Executive summary

An experimental and modelling approach was used to gain practical insights into how epidemics of myrtle rust (*Austropuccina psidii*) in New Zealand regions are affected by interactions between regional temperatures and rates of host and pathogen development. It focused on two key parameters influencing myrtle rust development:

Latent period, which is the time from infection to production of new spores. Latent development rate affects survival of the pathogen between infection events and between seasons. It was studied on two New Zealand natives (*Metrosideros excelsa* - pōhutukawa - and *Lophomyrtus* x *ralphii* - ramarama/rohutu hybrid) and on *Syzygium jambos* (rose apple), a highly susceptible species included for international comparison.

Leaf node emergence, which is a measure of the seasonal production of new plant tissue that is susceptible to *A. psidii* infection. It was studied on *M. excelsa* and *L.* x *ralphii* in seven New Zealand regions.

Latent period experiments were conducted under controlled temperatures at the Ecosciences Precinct in Brisbane (*M. excelsa* and *S. jambos*) and in the field in Auckland (*L.* x *ralphii*). We found a higher-than-expected temperature range for latent development on all three hosts (optimum 24–27°C). The minimum latent period was 5–6 days, which is much shorter than the 12 days previously reported. Negligible latent development occurred below 10°C. Modelling showed that winter mean air temperatures in the lower North Island and upper South Island of New Zealand can halt *A. psidii* development, enabling overwintering of *A. psidii* within plant tissues in colder areas. This means that re-infection during winter is not required for perpetuation between seasons. This is the first comprehensive study on this aspect of *A. psidii* biology and the findings suggest that latent period has a greater role in influencing myrtle rust development in New Zealand's temperate climate than previously thought.

Leaf node emergence on main shoots of *M. excelsa* and *L.* x *ralphii* was measured in field plots planted at seven sites from Auckland to Marlborough over two seasons. Data were used to develop a predictive model of regional leaf emergence rate in relation to accumulated temperature (thermal time). Emergence rates for *L.* x *ralphii* were about five times lower than for *M. excelsa* and the branching growth habit of *L.* x *ralphii* produces more side branches per tree, whereas *M. excelsa* produces fewer branches with more leaves per branch.

When emergence rates were compared in the northern North Island (Kerikeri) and northern South Island (Motueka), the current range of *A. psidii* establishment, rates for both species peaked in February and were minimum in July. Although the maximum emergence rate in summer for both species was similar in Kerikeri and Motueka, the peak was short-lived in Motueka. In Motueka, the winter-minimum emergence rate was zero for *M. excelsa*, but *L.* x *ralphii* still grew. There were differences in plant vigour caused by site factors, but both high and low vigour *L.* x *ralphii* plants had an optimum base temperature for thermal time accumulation of 0°C, whereas for *M. excelsa*, it was 6–8°C. This base temperature difference reflects the adaptation of *L.* x *ralphii* to cooler climates. The adaptation of *M. excelsa* to warmer climates was shown by the frost damage it suffered in the Hawke's Bay and Tasman plantings.

Opportunities for myrtle rust management. Interactions between leaf emergence rate and latent period were modelled for high- and low-vigour situations for each species in relation to regional temperature. Temperatures differentially favoured host growth over myrtle rust development for *L.* x *ralphii* in situations of high plant vigour, allowing the plant to continue growing as seasonal temperatures declined in autumn. Rust management may be achievable in cooler areas with a more temperate climate by fertilising and watering in autumn to encourage late season growth as declining temperatures become less favourable for rust development. Conversely, avoiding spring fertiliser applications could reduce risk by minimising the amount of susceptible plant tissue when warming temperatures in late spring favour rust development. These management suggestions are more likely to succeed in cooler southern and alpine areas and should be applied cautiously in warmer northern and coastal areas.

Further research is required to understand interactions between infection conditions, inoculum load and susceptibility of New Zealand host species so that the future effect of myrtle rust can be predicted as inoculum pressure in the environment increases. The latent period component of the Myrtle Rust Process Model needs to be updated to improve its accuracy in predicting myrtle rust risk in New Zealand regions.

Recommendations

1. The modelling approach used in this study has given a practical insight into how myrtle rust epidemics in New Zealand regions are affected by interactions between regional temperature and rates of host and pathogen development. It has identified some ways in which these interactions might be exploited for disease management. However, further development of management strategies requires the following additional aspects of myrtle rust epidemiology to be understood:

2. The interaction between infection conditions, inoculum load and genotypic susceptibility of New Zealand hosts. Myrtle rust inoculum pressure will increase as it becomes more widely established in the environment. Some host genera that have stood up well so far, like *Metrosideros, Leptospermum* and *Kunzea*, may become more severely affected over time. This has been reported in Australia for their hosts. We have the ability to predict this accurately in New Zealand, given appropriate experimental data and a modelling approach.

3. The requirement of susceptible young plant tissue for *A. psidii* infection makes host growth characteristics a key determinant of myrtle rust risk. To predict the effect of myrtle rust on a wider range of New Zealand and exotic species seasonal growth rates need to be characterised in conjunction with *A. psidii* development rates.

4. The new knowledge on latent period generated in this study needs to be used to update the Myrtle Rust Process Model to improve its accuracy. This model plays a key role in helping the understanding of myrtle rust development in New Zealand. It is now clear, given the higher-than-expected temperature requirement for *A. psidii* latent development, that latent period variation is much more important in determining regional risk of myrtle rust than previously thought.

1   Introduction

To better understand and predict the effect of myrtle rust on New Zealand native and exotic Myrtaceae we need to understand how the biology of *Austropuccinia psidii* interacts with the biology of its host plants and how both of these interact with the environment to influence regional myrtle rust epidemics. The biology of *A. psidii* is poorly understood compared with rust pathogens affecting many crop plants and this study aimed to help rectify that through controlled environment and field experiments linked to a modelling approach to give practical insights into drivers for myrtle rust development. The purpose was to allow more effective surveillance, accurately predict *A. psidii* geographic range and seasonal activity, predict effects on different host species and identify opportunities for management or control.

Two key aspects of pathogen and host plant biology are addressed in this study:

The asymptomatic period between infection and appearance of *A. psidii* symptoms. This interval, known in rust epidemiology as the latent period, is one of the main parameters determining the survival and rate of spread of rust pathogens. It needs to be understood in relation to host genotype, host phenology and environment factors. It is crucial for explaining seasonal disease development patterns and for developing disease management strategies.

The seasonal availability of young susceptible plant tissue is crucial to understand because *A. psidii* can only infect young, newly emerged leaves, stems, flowers and fruits. This means that host growth mediates when rust can infect its hosts.

The study focuses mainly on two New Zealand members of the Myrtaceae that are threatened by myrtle rust, *Metrosideros excelsa* and *Lophomyrtus* spp.

1.1   Seasonal variation in latent period

1.1.1   Background

Latent period is defined as the time between spore deposition and production of new uredinia containing spores (Teng & Close 1978). Rust latent periods are generally shorter on more susceptible hosts (Johnson 1980) and shorter at higher temperatures (Beresford & Mulholland 1987; Beresford & Royle 1988; Hernandez Nopsa & Pfender 2014). This study sought to accurately define how *A. psidii* uredinial latent period varies with temperature and leaf age for three Myrtaceae hosts, two from New Zealand and one from overseas:

Metrosideros excelsa 'Mini New Zealand Christmas Tree'; a highly susceptible pōhutukawa clone sourced from Australia.

Lophomyrtus x *ralphii* 'Red Dragon'; a highly susceptible horticultural selection of a natural hybrid between *L. bullata* (ramarama) and *L. obcordata* (rohutu).

Syzygium jambos (rose apple), which originates from Southeast Asia, is widely grown for its fruit and is extremely susceptible to myrtle rust (Tessman et al. 2001). A selection of unknown provenance from Australia was used.

1.1.2   Methods

The *M. excelsa* and *S. jambos* data were obtained at the Ecological Sciences Precinct controlled environment facility in Brisbane, Australia, and the *L.* x *ralphii* data were obtained from field inoculations at different times of year at The New Zealand Institute for Plant and Food Research Limited, Auckland, under permissions to work with *A. psidii* obtained from the Ministry for Primary Industries and the Environmental Protection Authority. Both controlled-environment and field inoculations used the pandemic biotype of *A. psidii* (Pegg et al. 2014) collected in Australia or New Zealand.

Controlled-temperature experiments on *M. excelsa* and *S. jambos*

A series of inoculations was made and for each inoculation four temperature treatments were provided by three constant temperature cabinets and a roof-top shade house with naturally fluctuating temperature (mean 18–25°C). For each temperature treatment and species, 10 replicate plants were spray-inoculated with a water suspension of 1 x 10^5 *A. psidii* urediniospores/ml. Infection was established with a 24-h wet period under a plastic tent in the dark at 18°C. The top 3–4 leaf pairs and internodes on each shoot were assessed daily to determine the time from inoculation to the first erupted uredinium (end of latent period).

Field experiments on L. x ralphii

The *L.* x *ralphii* 'Red Dragon' field inoculations were done in New Zealand on glasshouse-raised plants at different times of year (December to May) and symptom development and temperature were recorded in the field. For each inoculation, spores from field-infected plants were transferred to the top four leaves on four actively growing shoots. Inoculated shoots were misted with water and covered with plastic bags for 12–24 h. Symptom development was recorded daily, as described above, and temperatures were recorded with data loggers.

Modelling latent period response to temperature

The response of latent period (LP) for each leaf pair and associated stems (internodes) over the replicate shoots for each inoculation was modelled as the latent development rate, using the following function, which is a modification of a function used by Hernandez Nopsa & Pfender (2014) for wheat stem rust:

$$LDR = 24 * A * Temp * [Temp - Tmin] \text{ x } [1 - e^{\{B * (Temp - Tmax)\}}] \tag{1}$$

Where,

LDR =   Latent development rate (day^{-1})

A =   Rate of linear increase

Temp = Mean temperature during the LP

Tmin = Minimum temperature for latent development

Tmax = Maximum temperature for latent development

B =   Rate of exponential decrease

LP, therefore, = 1/LDR.

Parameters *A*, *Tmin*, *Tmax* and *B* were fitted to the LP and temperature data using the non-linear regression facility in Mintab® 18.1 (Minitab Inc., USA).

1.1.3   Results

For *M. excelsa* and *S. jambos*, the youngest 3–4 leaves visible at the time of inoculation developed *A. psidii* symptoms; older leaves were completely resistant. First symptoms (leaf distortion and red blotches) appeared 3–4 days earlier on the youngest leaves than the oldest symptomatic leaves, but the time to first uredinia was no more than 1 day earlier on the younger leaves and only in some inoculations.

For *L.* x *ralphii* the youngest two leaves visible at the time of inoculation, and the stem internodes between these, produced uredinia. While the first symptoms on leaves appeared as red spots, the first visible sign of stem infection was generally erupted uredinia. Latent period was not consistently different between younger and older leaves or between leaves and stems.

Irrespective of whether the latent period and temperature data points were derived from constant temperatures or from fluctuating shade house or field temperatures, they all lay along the same response curve for each host species, showing that mean temperature during the latent period is an excellent predictor of *A. psidii* latent period. The modelled response of latent period to temperature for all three species was U-shaped (Figure 1) and was shortest near the optimum temperature of 24–27°C and longest near the lower (8.7–10.6°C) and upper (32.4–33.6°C) limits. For *S. jambos*, the average minimum latent period was 5.2 days, for *L.* x *ralphii* 6.0 days and for *M. excelsa* 6.5 days. Latent period varied little between about 20°C and 28°C, but below 18°C, for *M. excelsa* and *L.* x *ralphii,* and below 15°C, for *S. jambos*, it increased markedly.

For *M. excelsa*, for which the widest range of temperature treatments was investigated, the increase in latent period at high temperature >30°C appeared to be caused by a direct adverse effect of the high temperature on the health of the host plants. At low temperature (<10°C) latent development was arrested, but infections were not killed. When one of the *M. excelsa* inoculations was held at a constant 8°C no symptoms developed after 1 month, but almost as soon as the temperature was raised to 12°C symptoms appeared, although uredinia were slow to develop at that temperature.
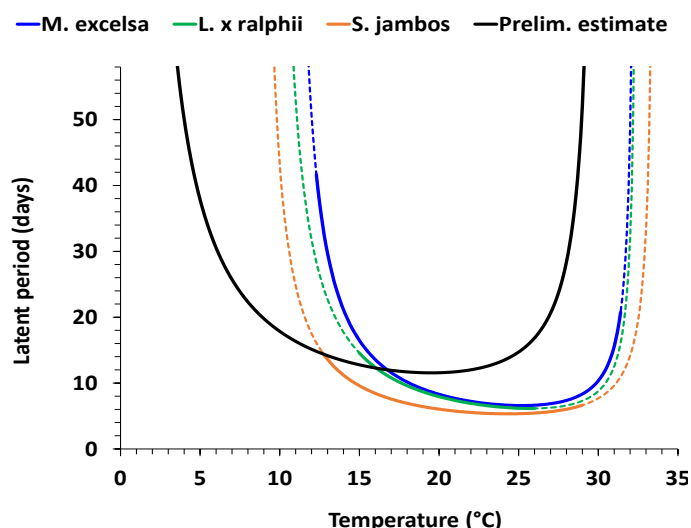
Figure 1. Modelled response of *Austropuccinia psidii* latent period to temperature for *Metrosideros excelsa*, *Lophomyrtus* x *ralphii* and *Syzygium jambos.* Dashed lines indicate data extrapolated beyond the experimental data points. The preliminary estimate was that used in the Myrtle Rust Process Model by Beresford et al. (2018).

1.1.4 Discussion

This study has, for the first time, accurately characterised the response of *A. psidii* latent period to temperature. The latent period response was substantially different from the preliminary estimate from literature used in the Myrtle Rust Process Model (Beresford et al. 2018). This study shows that the minimum latent period of 5–6 days is much shorter than the 12 days previously reported. It also shows that *A. psidii* latent development is favoured by higher temperatures than previously thought (optimum 24–27°C). Negligible latent development occurs below 10°C, which means that winter temperatures in the lower North Island and upper South Island of New Zealand will prevent *A. psidii* development. Upper North Island mean daily temperatures in summer are 18–22°C, which are below the optimum for latent development. This is an important finding because it indicates that latent period must have a greater role in influencing myrtle rust development in New Zealand than previously thought (Beresford et al. 2018).

Because latent development slows to a negligible rate below 10°C and resumes when temperatures rise again above about 12°C, it suggests that *A. psidii* will survive for long periods over winter within plant tissues in colder areas. This means that *A. psidii* does not require uredinial re-infection, or infection involving the telial stage, to perpetuate between seasons.

1.2 Myrtle rust development in relation to host growth

1.2.1 Background

Myrtaceae species vary in their genotypic susceptibility to *A. psidii* (Pegg et al. 2014) and relatively few are severely impacted. However, it became clear during the first year myrtle rust was in New Zealand that *Lophomyrtus* spp. (ramarama and rohutu) and *Metrosideros excelsa* (pōhutukawa) had substantial susceptibility and could be under threat (Beresford et al. 2018).

Even in the most susceptible genotypes, *A. psidii* can only infect young, newly emerged shoot, flower and fruit tissues. Leaves and stems develop ontogenic (developmental) resistance after emergence, which provides complete resistance to infection by the time leaves are fully expanded. It therefore follows that growth-mediated susceptibility causes seasonal fluctuations in disease risk by limiting infection to times of year when growth flushes occur. This is understood internationally, but only one previous study has attempted to quantify it (Tessman et al. 2001). Those researchers used the number of juvenile branches per tree as a causative variable when investigating factors associated with seasonal infection patterns.

In order to quantify this aspect of myrtle rust epidemiology for New Zealand hosts, we took a quantitative approach to model leaf emergence rates as an index of tree susceptibility. The method has been employed for modelling leaf canopy development in crops (Kirby et al. 1985; Clerget et al. 2008) and uses measurements of leaf emergence over time and regression against thermal time (accumulated temperature; °C days).

1.2.2   Methods

Leaf emergence was monitored on *Metrosideros excelsa* 'Vibrance' and *Lophomyrtus* x *ralphi* 'Red Dragon' on five trees of each species between September 2017 and April 2019 at each of the following seven Plant & Food Research sites (site codes indicated):

Mt Albert (Owairaka), Auckland	AK
Pukekohe, Franklin	PK
Te Puke, Bay of Plenty	TP
Havelock North, Hawke's Bay	HN
Palmerston North, Manawatu-Wanganui	PN
Motueka (Riwaka), Tasman	MO
Blenheim, Marlborough	BL.

The clonal trees were planted between September and October 2017. The PN plants were maintained in pots, re-potted in September 2018 and monitored only in 2018–19. Establishment of *L.* x *ralphii* in the first season was variable, which affected plant vigour at some sites. The *M. excelsa* plants at HN were frost damaged during winter 2018 and only the first year's data were used. Monitoring at MO was only done in 2017–18 because the *M. excelsa* plants were killed by frost during winter 2018 and the *L.* x *ralphii* were damaged by flooding in February 2018. Myrtle rust invaded the AK planting in the second season and monitoring was stopped in March 2019 because all the growing shoots on the two most vigorous plants had been killed. Myrtle rust was also found at PK in April 2019, but it did not interfere with the second season's monitoring. Myrtle rust did not appear at the other sites during the study.

Five shoots per tree on the five trees of each species at each site were monitored for the number of new nodes (leaf pairs) emerged every 2–4 weeks; more frequently during spring and summer and less frequently during autumn and winter.

1.2.3   Results

The number of leaf nodes emerged plotted against number of days since 1 July showed that emergence rates differed between the two species, as a result of their different growth habits (Figure 2). Emergence rate for *L.* x *ralphii* was about five times lower than for *M. excelsa* because the branching growth habit of *L.* x *ralphii* results in more side branches per tree, whereas *M. excelsa* produces fewer branches with more leaves per branch.

Emergence rate also differed between sites and seasons (site-years) because of site factors, including establishment success, rainfall, irrigation and fertiliser application. Within each species, the leaf emergence rate for each site-year was categorised as either high or low vigour.
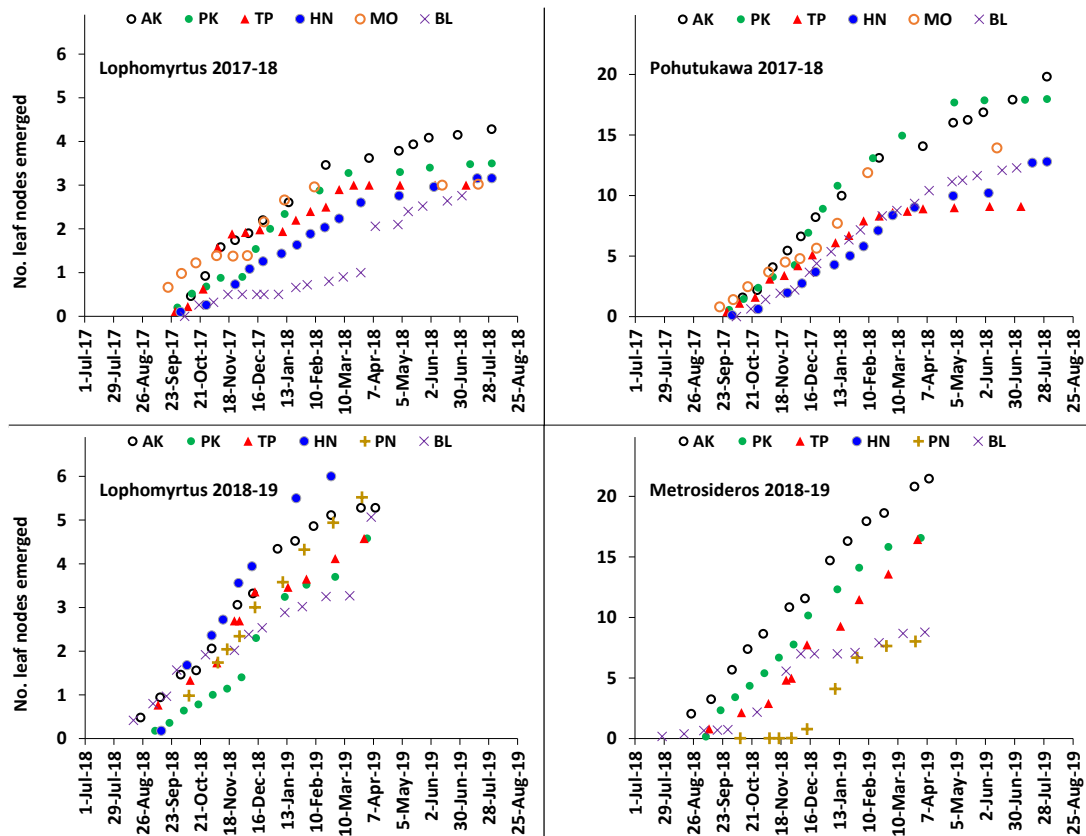
Figure 2. Emergence of stem nodes (leaf pairs) for *Metrosideros excelsa* and *Lophomyrtus* x *ralphii* at seven sites in 2017–18 and 2018–19. Sites were: Auckland (AK), Pukekohe (PK), Te Puke (TP), Havelock North (HN), Palmerston North (PN; 2018–19), Motueka (MO; 2017–18) and Blenheim (BL).

For the high- and low-vigour categories separately, leaf emergence was plotted against thermal time (accumulated temperature in °C days) above base temperatures from 0–15°C. The optimum base temperature for explaining leaf emergence across all sites for each species was that at which no leaf nodes emerged when no degree days accumulated, i.e. the base temperature giving a regression intercept closest to zero (Figure 3). The optimum base temperature differed substantially between the two species but not within each species for low- or high-vigour site-years. For *L.* x *ralphii*, the optimum base temperature was 0°C and, for *M. excelsa*, it was 6–8°C.
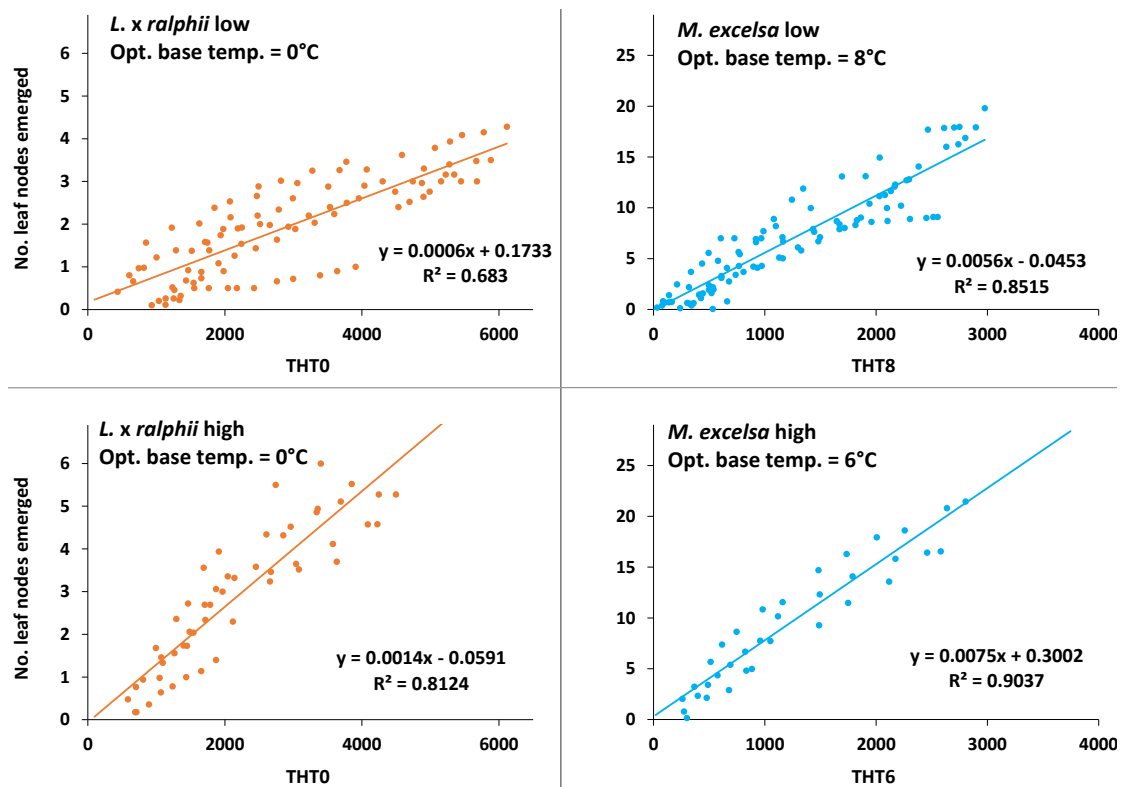
Figure 3. Leaf node emergence modelled using thermal time (°C days) above various base temperatures (THT) for _Metrosideros excelsa_ and _Lophomyrtus_ x _ralphii_ at seven sites in 2017–18 and 2018–19. Site-years were categorised as either low or high vigour.

1.2.4   Discussion

The low- and high-vigour categories of leaf emergence rate for each species reflected a range from plants that hardly grew during a season to those that grew at close to the maximum rate for the species. The consistent difference in the optimum base temperature between the two species, irrespective of vigour, reflects differences in their climatic adaptation; _L._ x _ralphii_ is adapted to colder climates and _M. excelsa_ to warmer climates. _M. excelsa_ was also very frost tender, as evidenced by the frost damage it suffered at HN and MO.

The slope of the leaf node versus thermal time regression lines gives the average leaf emergence rate for a season. Although this averages over short-term growth flushes and ignores the effect of plant architecture on amount of susceptible tissue, it does provide an index of vulnerability to myrtle rust attack. When coupled with seasonal temperature (thermal time) and latent period it is possible to predict the relative risk of myrtle rust development in plant populations in different regions, as detailed below.

1.3　Modelling species vulnerability

1.3.1　Background

The effect of regional temperatures on the interaction between latent development and leaf emergence was modelled to identify whether some regions and seasons might favour plant growth over myrtle rust development. The finding that leaf emergence for *L. x ralphii* can occur down to 0°C, whereas for *M. excelsa* it cannot occur below about 7°C, and that latent development of *A. psidii* cannot occur below 10°C on both species suggests that such situations might exist.

1.3.2　Methods

Both the temperature dependent processes of leaf node emergence and *A. psidii* latency were stated as developmental rates, i.e. the proportion of each process completed each °C day. The emergence rate is the slope of the leaf emergence-thermal time regression and the latent development rate is the modelled LDR (Equation 1).

These parameters were used with temperature data from Kerikeri and Motueka, which represent the northern and southern geographic limits of the myrtle rust range in New Zealand at the time of writing (6 May 2019). The slowest and fastest leaf node emergence rates for each species were used to represent high- and low-vigour situations.

1.3.3　Results

Emergence rates for both species peaked in February and were minimum in July (Figure 4). Although the maximum emergence rate in summer for both species was similar in Kerikeri and Motueka, the peak was short-lived in Motueka. Minimum rates were substantially lower further south because of the colder winter temperatures. In Motueka, the winter-minimum rate was zero for *M. excelsa*, but not for *L. x ralphii*.

For *L. x ralphii* with high vigour in Kerikeri and Motueka, there was a period during winter and early spring (June–September) when latent development was slower than leaf emergence. The difference was much greater and more extended in Motueka because temperatures <10°C, which inhibit rust development, were more frequent.

For *M. excelsa*, the opportunity for the plant to out-grow the rust arose in the high-vigour situation in Kerikeri, between July and September. Field temperatures allowed the differential between the 7°C limit for leaf emergence and 10°C limit for latent development to become important.

For the low-vigour situation in both species, emergence rate during winter was so low there was no opportunity for leaves to emerge when rust was not developing. However, little rust would develop on such low-vigour plants anyway, because negligible susceptible tissue would be produced.
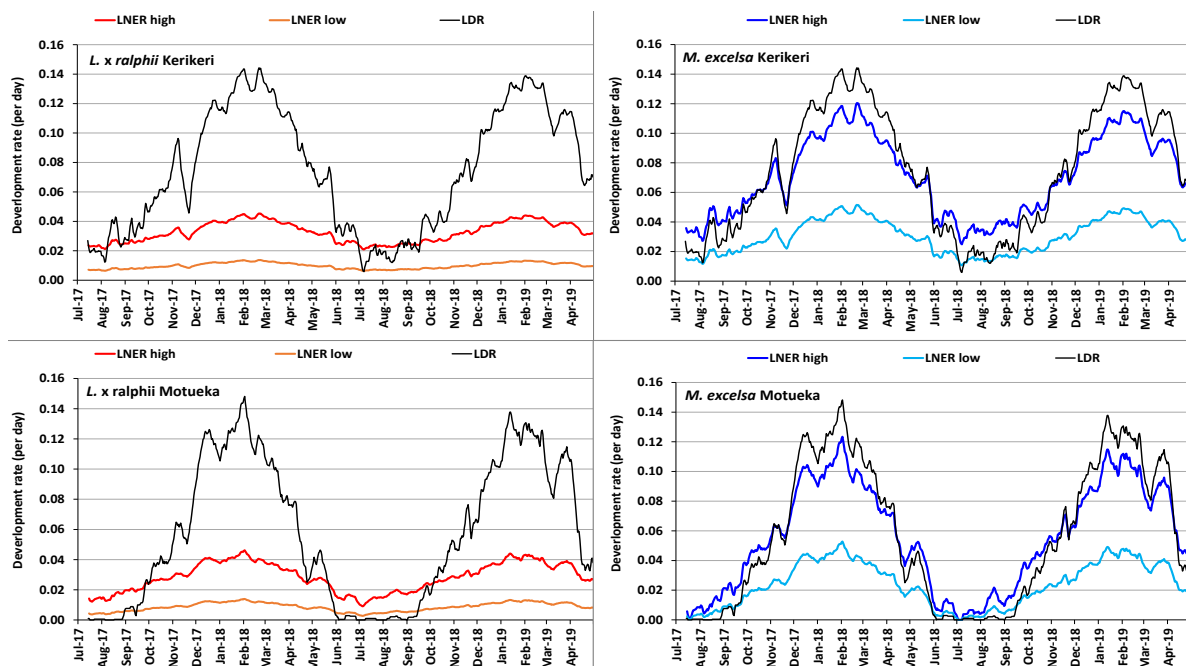
Figure 4. Modelled daily development rates at Kerikeri and Motueka between July 2017 and April 2019 for leaf node emergence rate (LNER) in high- and low-vigour situations for *Lophomyrtus* x *ralphii* and *Metrosideros excelsa* and for latent development rate (LDR) for *A. psidii*. LDR graphs at a site are the same for both host species.

1.3.4   Discussion

The likelihood that temperature could differentially favour host growth over *A. psidii* development, allowing the host to out-grow the pathogen, would be greatest for *L.* x *ralphii* plants with high vigour in areas with winter mean temperatures below 10°C. Such temperatures occur in coastal northern South Island and southern North Island areas and at higher altitudes. For *M. excelsa*, the smaller differential between the minimum temperatures for growth (7°C) and *A. psidii* latent development (10°C) means there is a lower likelihood of the host out-growing the pathogen.

In managed Myrtaceae plantings in New Zealand, risk of myrtle rust damage to species that are cold-temperature adapted may be reduced by fertilising and watering in autumn to encourage late season growth as temperatures decline and become unsuitable for *A. psidii* development. Conversely, avoiding fertiliser applications in spring could reduce risk by minimising the amount of susceptible plant tissue when warming temperatures in late spring favour rust development. Similarly, to reduce risk to young trees being planted for amenity purposes or site restoration, planting should be done in autumn while temperatures are still warm enough for growth, but are becoming too cold for the pathogen. This approach would be helped by removal of any *A. psidii* inoculum on existing Myrtaceae hosts in the area being planted. These disease management suggestions based on differential temperature responses for host and pathogen development are most likely to succeed in cooler southern and alpine areas where development of the pathogen is likely to be arrested by cool winter temperatures. They should be applied cautiously in warmer northern and coastal areas and are probably not appropriate in subtropical or tropical climates. They would apply less to high-temperature adapted Myrtaceae species.

In the natural estate, plant vigour for these and other Myrtaceae species is generally likely to be low because of low soil fertility, shading by other plants and periodic water stress. In this situation myrtle rust is likely to be very damaging on susceptible hosts, especially in environments with high relative humidity. Although the small amount of young susceptible tissue

available most of the time will mean myrtle rust is not often be noticeable, whenever temperatures are warm enough for new shoots to emerge, *A. psidii* , if present, will readily infect them.

This study has not considered the effect of pathogen multiplication via infection and re-sporulation on the interactions between pathogen and host development rates. Even if winter rates of rust development were slower than rates of leaf emergence, rust could still have a devastating effect if infection and sporulation rates are high when temperatures increase in spring. Infection and sporulation processes are driven by moisture, temperature and host susceptibility. How these processes, relative to the host growth and pathogen development processes addressed in this study, interact in the New Zealand environment needs to be understood through further research.

2   Acknowledgements

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