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Title: Myrtle rust impacts on a wet sclerophyll forest in the Tallebudgera Valley, South-East Queensland.

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1. Executive Summary

Introduced non-native pathogens can impact on the health of trees and alter the fundamental way that forest's function. Tree assemblages play vital roles in structuring the micro-climate of forests, thereby influencing how they function and the services the forest provide. The Myrtaceae fungal pathogen *Austropuccinia psidii*, commonly known as myrtle rust, was first reported in Australia in 2010 and has since spread to more forests, including wet sclerophyll forests of South-East Queensland. The community level impacts of *A. psidii* have yet to be investigated with studies generally taking a population level approach. In this project, likely responses of a wet sclerophyll forest (Tullebudgera) to repeated infestation were assessed by tracking mortality rates of five locally abundant Myrtaceae tree species in the forest mid- and understory; and comparing vegetation % cover in plots that showed high rates of mortality and plots with little signs of mortality. Canopy gap fraction was used as a surrogate measure of light availability to assess the implications of this mortality effect on community structure, vegetation cover, recruitment, and soil nutrients. Myrtaceae species that showed low levels of mortality in 2016 had declined considerably by 2019 (e.g., *Archirhodomyrtus beckleri* and *Decaspermum humile* decreased by 72% and 45%, respectively). Plots with more light gaps due to *A psidii*-induced tree mortality tended to be positively correlated with increased cover of non-native species in the understory (e.g., *Lantana camara* and *Ochna serrulate*). After just four years of studying the site, we found significant changes to light conditions and forest understories as well as a rapidly increasing mortality rates for species that originally showed little mortality. One species that was highly impacted, *Archirhodomyrtus beckleri*, showed a significant shift in specific leaf area (SLA) between infected populations and uninfected populations, while a less impacted species, *Acmena smithii* did not. Specific leaf area is commonly used as an indicator of a plant's growth strategy. Although only for two species and needing more research, the changes in SLA suggest that the species with a higher mortality rate has a higher SLA (and therefore a resource acquisition specialist) when not infected than a species that shows little mortality. Relative values of SLA and the plasticity of SLA may be surrogate measure for susceptibility to *A. psidii,* but further research is needed. These early changes in canopy gaps, species composition and vegetation cover will likely become more pronounced over time. Follow-up studies are highly recommended to document the pathogen impacts on populations and community dynamics so that timelines of impact can be better understood, and interventions optimised.

2. Introduction

Introduced non-native pathogens have the capacity to change fundamental vegetative structures of forests by killing foundational tree species, often resulting in a significant shift in the function of such ecosystems (Needham et al., 2016; Mack et al., 2000; Loo 2009). Studies have shown that wet sclerophyll forests of South East Queensland (SEQ) are already under significant threat from tree clearing for urban development (Mogilski et al., 2020). Since 2010, myrtle rust was identified in Australia and it poses a significant threat to wet sclerophyll forests where Myrtaceae trees dominate the different canopies and strata of these forests (Carnegie et al., 2015, 2018; Pegg et al., 2014, 2017). In places where similar forests have been attacked by introduced non-native pathogens, the death of foundational tree species has resulted in encroachment of non-native species that were not previously occurring in those systems (Jones et al., 1997; Lovett et al., 2006; Moles et al., 2008; MacDougall et al., 2009). Usually, colonization by invasive species and consequent loss of biodiversity may mean that the system is transformed permanently. The Tallebudgera wet sclerophyll forest in SEQ is dominated by Myrtaceae species in the layers of its canopy and has been infected by the myrtle rust pathogen, *Austropuccinia psidii* since 2011 (Figure 1-2). In 2014, a long-term study was established to track the effect of repeated infection by *A. psidii* on key Myrtaceae tree species while also documenting the changes in the function of the forest.

Figure 1 *Rhodamnia maideniana* leaves (a), branches (b), flowers and fruits (c) infected by *A. psidii* at Tallebudgera (Photo credit: Geoff Pegg).

Figure 2 Canopy opening (a) as a result of myrtle rust induced dieback of foundational Myrtaceae species and dieback levels in the midstory (b) of the Tallebudgera forest (Photo credit: Geoff Pegg).

The current project addresses some aspects of the long-term study by documenting and tracking mortality of the dominant Myrtaceae species at Tallebudgera (Pegg et al., 2014, 2017). The project also analyses how the understory vegetation changes in response to Myrtaceae species mortalities. The project also assesses whether there are any systematic shifts in response to repeated infection by the myrtle rust pathogen using a commonly used leaf trait, specific leaf area (SLA). Sclerophyllous leaves are known to be tough and leathery, with a relatively low SLA – indicating high investments in leaf construction and carbon assimilation (Specht 1969; Ackerly et al., 2001; Vendramini et al., 2001). These traits are associated with long leaf survival, nutrient retention, and protection from desiccation (Mooney and Dunn 1970). SLA, a trait that measures the ratio of leaf area to its dry mass (SLA = Leaf area/Leaf dry mass; Connelissen et al., 2003; Paz-Dyderska et al., 2020) is an important plant trait that has been used in numerous ecological studies as a surrogate measure of the resource investment a plant makes in constructing leaf tissue, a key site of carbon assimilation, water relations and energy balance in a plant (Osunkoya et al., 2010; Reich et al., 2003; Leishman et al., 2007). SLA drives the amounts of carbon accumulated per unit energy invested by the plant (Reich et al., 2003; Wright et al., 2004).

3. Aim

a) To determine the mortality of five locally abundant populations of Myrtaceae species over time – *Acmena smithii, Archirhodomyrtus beckleri, Decaspermum humile, Gossia hillii and Rhodamnia maideniana* in response to repeated infection by *A. psidii* at the Tallebudgera site in SEQ.

b) To assess the effect of tree mortality/death on canopy transparency by quantifying canopy gap fraction in plots that show evidence of *A. psidii* induced tree mortality and those without evident mortality.

c) To assess the effect of mid-story tree mortality on the understory vegetation % cover (both native and non-native vegetation), seedling composition and soil nutrients.

d) Finally, we tested whether there was a detectable shift in functional traits of two of the five Myrtaceae species as a result of infection by *A. psidii*. We used the SLA trait because it has been found to be a surrogate measure of how plants allocate energy and biomass to their leaves. We compared SLA from the TRY database (control) and SLA from the field (treatment) where trees have been infected by *A. psidii*.

4. Methods

Study Design – We resampled long term plots that were established by Pegg et al. (2017.) Pegg et al. (2017) set up four 50m by 2m transects were delineated at the Tallebudgera Valley Study site. Each transect was divided into 100 plots, making a total of 400 plots for the four transects. The following measurements were then made along the four transects:

a. **Mortality/Death of the five targeted Myrtaceae species** (*Acmena smithii, Archirhodomyrtus beckleri, Decaspermum humile, Gossia hillii and Rhodamnia maideniana*) – Presence of trees belonging to these species were recorded along the transects, including the number of dead trees/species in the different forest layers. These measurements were conducted in 2017 and 2019, and the results were compared with similar measurements that were taken in 2016 by a different study (Pegg et al., 2017). This was to enable the study to have a time series comparative framework of survivorship and mortality of key Myrtaceae species in response to repeated infection by *A. psidii*.

b. **Canopy gap fraction (CGF) index**, which is a measure of the percentage of light passing through the canopy without encountering foliage, was measured in each plot in the four transects. The CGF was then correlated with *A. psidii* induced tree mortality (a above).

c. **Vegetation analysis**: To assess the impact of myrtle rust on the composition of the understory, ground vegetation cover percentage and seedling composition were measured in each plot – those with *A. psidii* induced tree mortality and those without evidence of mortality. Percentage vegetation cover was divided into native and non-native plant species for each plot in all four transects.

d. **Soil chemical analysis and specific leaf area (SLA) measurements** – Soil samples were collected from the study plots and analysed for soil carbon, nitrogen, ammonium, nitrate, phosphorus, potassium, and sulphur in plots with and without tree mortality. This was to identify any changes in soil elements or nutrients as a response to tree mortality caused by *A. psidii* infection.

To test whether there is a detectable shift in growth strategies as a result of infection by *A. psidii*, SLA was measured for two of the five targeted Myrtaceae species – *Acmena smithii* and *Archirhodomyrtus beckleri*. The two species were chosen because they had SLA data available from the TRY database and also from the field (TRY Plant Trait Database, https://www.try-db.org/TryWeb/Home.php - Kattge et al., 2020). Therefore, their SLA data from the field (infected) were compared with SLA data from TRY (uninfected/control). SLA is widely used as a surrogate measure of how plants allocate energy and resources to their leaves. Leaf area was measured on 50 leaves per species, and then leaves were dried before measuring their dry mass to calculate SLA (Leaf area/Leaf dry mass).

5. Results

Key Results from the Investigations

a. Tree mortality, survivorship and canopy gap fraction (CFG) in response to repeated infection by *A. psidii*

There was a significant decline in Myrtaceae tree survivorship from 2016 to 2019 as the death rate increased for most of the species investigated in this study [Survivorship trends for all Myrtaceae species were as follows: 2016 (96%) > 2017 (80%) > 2019 (45%)]. Statistical analyses showed that there were significant differences in the death rates of the five focal species of the study – with *Archirhodomyrtus beckleri* showing the highest deaths in 2019 (88.4%), followed by *Decaspermum humile* (83.3%), *Gossia hillii* (34.5%) and *Rhodamnia maideniana* (20.9%). Meanwhile, *Acmena smithii* had the lowest death rate (4.82%) in response to repeated infection by *A. psidii*. CGF was significantly higher in plots that showed tree deaths than those without evidence of tree deaths as a result of *A. psidii* infection, which means that the deaths of mid-story trees from myrtle rust resulted in higher canopy transparency.

b. Effect of tree mortality on vegetation cover, community composition and soil nutrients

Because there was a significant positive relationship between tree deaths and CGF, a decision was made to henceforth use CGF as a surrogate measure of *A. psidii* induced tree deaths/mortality. One key finding of the effect of tree deaths on vegetation cover was that as CGF increased, native vegetation % cover declined while non-native vegetation % cover significantly increased. The non-native vegetation that replaced the dead Myrtaceae species included Weeds of National Significance (WoNS) like *Lantana camara* and other invasive species according to the Queensland Biosecurity Act of 2014 as shown in Table 1.

There was no discernible difference between plots where there was tree mortality and those without mortality in terms of the number of seedlings in the regenerative strata of the forest. However, there was a relative increase in *A. smithii* seedlings by 14% between 2016 and 2019; and a 1% increase for *G. hillii* while there was an 8% decline in *A. beckleri* seedlings within the same time period, followed by *D. humile* (4%) and *R. maideniana* (2%). There was no difference in soil nutrients (e.g., nitrogen, carbon, ammonium, nitrates, phosphorus) between plots with evidence of tree deaths and those with no evidence of tree deaths.

Table 1 Species composition in the regenerating area of the forest, showing only plants under 1 m in height. Plants were categorized as either native or non-native, and some of the non-native plants were identified as invasive species according to the Queensland Government guidelines.

Species, Family	**Category/Vegetation type**	**Life form**	Distribution
Acmena smithii, **Myrtaceae**	Native, Rainforest/wet sclerophyll	Tree	33 plots
Aphananthe philippinensis, ***Cannabaceae/Ulmaceae***	Native, Rainforest	Tree	7 plots
Archirhodomyrtus beckleri, **Myrtaceae**	Native, Rainforest	Tree	7 plots
Beilschmiedia elliptica, **Lauraceae**	Native, Rainforest	Shrub	1 plot
Decaspermum humile, **Myrtaceae**	Native, Rainforest	Tree	6 plots
Ficus coronata, **Moraceae**	Native, Rainforest, open country, wet sclerophyll,	Tree	1 plot
Flindersia australis, **Rutaceae**	Native, Rainforest, dry scrub	Tree	1 plot
Gossia hillii, **Myrtaceae**	Native, Dry rainforest, subtropical rainforest	Shrub	3 plots
Guioa semiglauca, **Sapindaceae**	Native, Rainforest	Tree	3 plots
Hibbertia dentata, **Dilleniaceae**	Native, Open forest, rainforest edge	Herb	3 plots
Jagera pseudorhus, **Sapindaceae**	Native, Rainforest	Tree	3 plots
Macaranga tanarius, **Euphorbiaceae**	Native, disturbed rainforest areas	Tree	7 plots

Polypodiopsida, **Ferns**	Native, rainforest	Shrub/Herb	75 plots
Rhodamnia maideniana, **Myrtaceae**	Native, wet sclerophyll forest	Shrub	7 plots
*Ageratina adenophora**, **Asteraceae**	Non-native, toxic, allelopathic	Shrub	5 plots
*Ageratina riparia**, **Asteraceae**	Non-native, toxic	Herb	11 plots
*Ageratum houstonianum**, **Asteraceae**	Non-native, pastures, moist forest clearings, toxic to livestock	Herb	6 plots
Ardisia crenata, **Primulaceae**	Non-native, Rainforest	Shrub	16 plots
Cinnamomum camphora, **Lauraceae**	Non-native, riparian zones, rainforest edges	Tree	8 plots
*Paspalum mandiocanum**, **Poaceae**	Non-native, wet forests, pastures	Herb	22 plots
*Erigeron canadensis**, **Asteraceae**	Non-native, temperate zones,	Herb	1 plot
*Lantana camara**, **Verbenaceae**	Non-native, variety of environments	Shrub	1 plot
*Ochna serrulata**, **Ochnaceae**	Non-native, open sunny areas, dry forests	Shrub	13 plots
Psychotria sp., **Rubiaceae**	Non-native, various environments	Shrub	3 plots
Setaria palmifolia, **Poaceae**	Non-native, temperate and tropical forests	Herb	13 plots

***Invasive species according to the Biosecurity Act of 2014**

c. Change in SLA as a response to pathogen infection

Archirhodomyrtus beckleri: There was a significant difference between SLA trait values from the infected sites and SLA trait values from the control plants, i.e., values obtained from the TRY database for this species (Figure 1 below). The SLA values obtained from the infected field study site showed that the plants invested less on the leaf biomass, hence high SLA when compared to uninfected plants. It is interesting to note that this species depicted high levels of susceptibility to *A. psidii* as evidenced by high mortality rates after about five years of repeated infection (5a, above).

Acmena smithii: In this species, there was no significant difference in SLA trait values between plants under attack by the myrtle rust pathogen and those from the TRY database. Coincidentally, *A. smithii* appears to be more tolerant to *A. psidii* with less mortality recorded despite infections occurring repeatedly over a five-year period (5a, above).

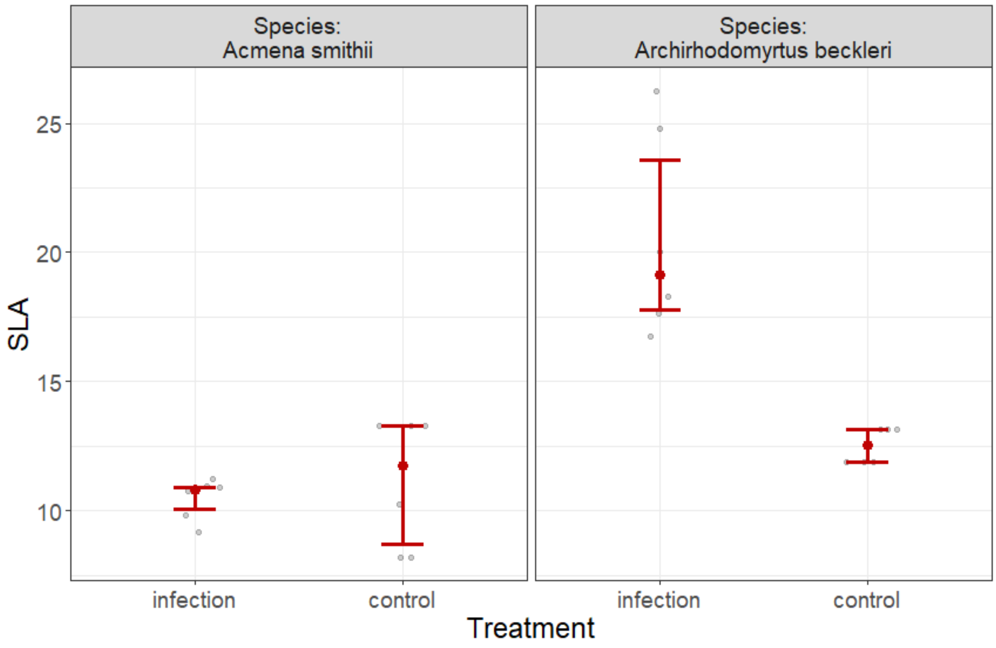

Figure 1 Variance in specific leaf area (SLA) between *A. psidii* infected sites (Tallebudgera valley study site) and non-infected sites (sourced from the TRY Plant Trait database, Kattge et al.,2020) for two focal species in this study, i.e., *Acmena smithii and Archirhodomyrtus beckleri*.

6. Discussion and Conclusion

The outcome of this project has contributed to better understanding of the effects of *A. psidii* on the plant community ecology of the Tallebudgera wet sclerophyll forest. The pathogen-induced tree mortality of key mid-story Myrtaceae species resulted in significant canopy transparency that altered the micro-climate in the understory of the forest. The high mortality rates of species such as *A. beckleri, D. humile* and *R. maideniana* means that there is a significant change in species composition in this wet sclerophyll forest at Tallebudgera. Although *A. smithii* showed less mortality, the few mortality events recorded in this species were among the regenerating stage as was also observed for *R. maideniana*, implying a possible decrease in recruitment for these species at the study site. Over time, this trend could have significant negative effects for forest function.

The alteration of the micro-climate in the understory because of the death of the mid-story Myrtaceae species resulted in an increase in the recruitment of more non-native vegetation cover than native vegetation cover. Of the 11 non-native species recorded in the forest floor, seven are invasive species that are otherwise absent in undisturbed wet sclerophyll forests, including *Lantana camara*, a Weed of National Significance (WoNS) in Australia (https://weeds.org.au/, accessed: March 2021). Other non-native light tolerant species that are considered invasive species in Queensland that were recruited as a result of canopy openings were *Ochna serrulata* and *Ardisia crenata* (Queensland Government, 2020). Previous studies have found that vegetation diversity and species richness were inversely proportional to increasing lantana cover (Stohlgren 2002; Raghubashi and Tripathi 2009). Therefore, colonization of the Tallebudgera wet sclerophyll forest by non-native invasive species could be detrimental to the persistence of the ecosystem, and possibly result in an increase in invasive species.

A higher SLA for pathogen infected *A. beckleri* populations could signal a trade-off for resources from leaf construction to defense against the pathogen, resulting in less dense leaves. On the other hand, because of high tolerance to the pathogen, *A. smithii* SLA patterns did not change with increase in infection. Sclerophyllous leaves are known to be tough and leathery, with a relatively low SLA – indicating high investments in leaf construction and carbon assimilation (Specht 1969; Ackerly et al 2001; Vendamini et al., 2001). These traits are associated with long leaf survival, nutrient retention, and protection from desiccation (Mooney and Dunn 1970). Previous studies have shown that high SLA plants focus on less costly tissues and generally expend less investment on defense against enemies (Endera and Coley 2011; Parker and Gilbert 2018; Coley et al., 1985; Welsh et al., 2016). There is evidence that suggests that pathogens can potentially regulate long term functioning of plants, which means that functional traits like SLA could be a key indicator of susceptibility to pathogens (Fahey et al., 2020). Therefore, the fact that in this study, particularly for the high mortality species (*A. beckleri*), SLA changes with infection but less so for the low mortality species (*A. smithii*) implies that the pathogen impacts represent changes in how the leaves are produced, thus impacting on the ability of the species to photosynthesize. This could mean that *A. psidii* is changing how species produce leaves, whether they produce larger leaves with less tissue or smaller leaves with more tissue to fortify their defenses (Peirez-Harguindeguy et al., 2013; Wright et al., 2004; Cappelli et al., 2020). However, because only two species had corresponding data from the field (*A. psidii* infected) and from TRY database (control), we suggest a more comprehensive investigation of leaf traits, including SLA on both infected and uninfected populations of the focal species in order to draw stronger conclusions on the effects of this pathogen on resource allocation.

7. Recommendations

Despite *A. psidii* presence and monitoring at the study site having only occurred in the short-term (~5 years), significant changes in community structure indicate that with time these impacts may become more pronounced. Thus, a continuous monitoring program is highly recommended to document population and community changes from the impacts of *A. psidii* in the Tallebudgera valley and similar sites across Eastern Australia where the pathogen occurs. Follow-up studies that record the impact of *A. psidii* on ground cover composition, soil nutrients and recruitment in the forest are necessary to identify the long-term impacts of this pathogen on the community ensemble of the Tallebudgera wet sclerophyll forest.

The current project has focused on the impact of *A. psidii* on populations of five dominant Myrtaceae species in the under-to mid-story at the study site, i.e, *Acmena smithii, Archirhodomyrtus beckleri, Decaspermum humile, Gossia hillii,* and *Rhodamnia maideniana*. Further assessment is necessary to investigate the effect and extent of impact of *A. psidii* infection on other Myrtaceae species that are present in the forest overstory, e.g., *Eucalyptus grandis* and *Lophostemon confertus* (Pegg et al., 2017).

An additional more comprehensive investigation of the impacts of *A. psidii* on SLA and other important functional traits is highly recommended, focusing on both infected and uninfected populations of a range of Myrtaceae species. This will enable us to draw stronger conclusions about the possible effects of this pathogen on plant energy investment strategies in these species. If a strong relationship can be established between SLA (and possibly other functional traits) and infection by *A. psidii* for a range of species exhibiting different levels of susceptibility, SLA [a soft trait that is easier to measure] could then be used as a surrogate predictor of plants' susceptibility to the pathogen.

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