IMPACTS OF THE INVASIVE PATHOGEN *AUSTROPUCCINIA PSIDII* (MYRTLE RUST) ON AUSTRALIAN NATIVE COMMUNITIES

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For my grandma Chabela and my friend Roy

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ABSTRACT

Despite ongoing research into the invasion of the fungal pathogen *Austropuccinia psidii* (myrtle rust) in Australia, few studies have attempted to examine the impacts it has on natural native communities at both the species- and community-level. This is surprising considering that it infects the Myrtaceae, one of the dominant plant families in Australia. Furthermore, the lack of a national program collating data on *A. psidii* spread, hosts and impacts makes restoration and conservation decision making challenging for natural resource managers. Therefore, the overarching aim of this thesis was to determine the impacts of the invasive pathogen *Austropuccinia psidii* on Australian native vegetation communities.

Specifically, this thesis determines the geographic extent and impacts of *A. psidii* on Australian native vegetation communities from the outcomes of a survey distributed to researchers, land managers and government employees (*Chapter 2*); the susceptibility of 24 previously untested species/sub-species with a particular emphasis on endangered species (*Chapter 3*); the impacts of *A. psidii* on plant architecture, growth and biomass allocation of susceptible species after fire under controlled glasshouse conditions (*Chapter 4*); and finally the indirect and direct impacts of *A. psidii* on community species richness and abundance through a large scale field experiment (*Chapter 5*).

Altogether, this suite of studies represents a significant contribution to our understanding of the *A. psidii* invasion in eastern Australian vegetation communities. The outcome of this contribution will hopefully be to inform and assist the successful conservation of one of the most iconic plant families in Australia as well as the communities that they define.

CERTIFICATE

This thesis constitutes an original contribution and has not been submitted, in any form, for a higher degree at any other university or institution.

All research in *Chapter 2* was conducted with the approval of the Faculty of Science and Engineering Human Research Ethics Sub-Committee at Macquarie University (Reference number: 5201500739).

The work of others has been used to prepare some aspects of the thesis and the extent of their contribution is clearly outlined below. Katherine Berthon (RMIT University, Melbourne) elaborated the map presented in *Chapter 2* and collaborated with running the glasshouse experiment and writing the manuscript which is presented in *Chapter 3*. Dr Karanjeet Sandhu performed the inoculation of plants in *Chapter 2*. Dr Saskia Grootemaat (NSW Fire Service) elaborated the map included in *Chapter 5*. All other aspects of the work presented in this thesis were done by me.

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CHAPTER ONE

Introduction

Pathogens are powerful evolutionary forces that can shape the population dynamics of individual species and the structure and function of the communities in which they occur (Burdon et al. 2006). Plant pathogens in particular are important drivers of species competition, succession and extinction in natural plant communities (Fisher et al. 2012; Chakraborty 2013). When plant pathogens are introduced to new regions, most usually through anthropogenic activities, they can become invasive and have significant impacts on species and communities, representing a threat to commercial production and biodiversity (Dobson and Crawley 1994; Ayres and Lombardero 2000; Gilbert 2002; Ellison et al. 2005; Loo 2009; Roy et al. 2014).

Among the various groups of pathogens which infect plants, fungal pathogens are regarded as the most important in terms of potential impacts (Van Alfen 2001). The largest order of fungal plant pathogens are the Pucciniales (Fungi, Basidiomycota, Pucciniomycotina) (Tavares et al. 2014). The disease caused by these fungi is referred to as "rust" given that the typical symptom is "rust-coloured" (orange or yellow) pustules that form on any above-ground organ but usually on leaves (McKenzie 1998). The rusts are ecologically obligate biotrophic parasites, depending entirely on living host cells to complete their biological cycle (Cummins and Hiratsuka 2003). They are best known worldwide for infecting highly valuable crops. For example, *Puccinia graminis* (wheat rust) has decimated crops and put food security at risk since ancient Roman times (Schumann 1991). However, there are fewer cases where rust fungi are known to jeopardize natural vegetation communities. One famous example is *Cronartium ribicola* (white pine blister rust), which infects several needle pine species in North America (Colley 1918; Geils et al. 2010), causing tree mortality as well as related cascade community effects such as changes in hydrology, fauna population dynamics and species succession

(MacDonald and Hoff 2001; Ellison et al. 2005). Another example that is currently affecting natural vegetation globally is *Austropuccinia* (=*Puccinia*) *psidii*, which is the focus of this thesis.

The study species: *Austropuccinia psidii*

Austropuccinia psidii is a pathogenic fungus native to South and Central America that affects species in the Myrtaceae family (Winter 1884; Coutinho et al. 1998; Glen et al. 2007; Beenken 2017). It causes a disease known as guava, eucalyptus, ohia or myrtle rust, depending on the species or group of species that it affects in different countries. Symptoms can vary from spots or flecks in resistant species (Fig. 1a), to characteristic yellow urediniospores (Fig. 1b) that can lead to leaf distortion (Fig. 1c), defoliation (Fig. 1d), branch dieback, reduced reproductive output (Fig. 1e) and even death (Fig. 1f) in the most susceptible species (Smith 1935; Rayachhetry et al. 2001; Uchida et al. 2006; Carnegie et al. 2016). Of the five species of pathogenic rust fungi that are known to infect plants in the Myrtaceae family (*A. psidii*, *Phakopsora rossmaniae*, *Physopella jueli*, *P. xanthostemonis*, *Puccinia cygnorum*) (Simpson et al. 2006), none have been as devastating in their impact or have such a widespread distribution as *A. psidii*.

Fig. 1 Symptoms of *Austropuccinia psidii* infection on different species, life stages and tissues **a)** *Eucalyptus viminalis* seedling showing necrotic flecks (hypersensitive reaction) on leaves, **b)** yellow pustules on a leaf of *Rhodamnia rubescens*, **c)** leaf distortion in *R*. *rubescens*, **d)** defoliation of *R*. *rubescens*, **e)** reduced reproductive output in *Chamelaucium uncinatum* through infection of flowers and **f)** mortality of *R. rubescens* trees after repeated infections.

The number of *A*. *psidii* hosts has increased dramatically with its rapid global spread, with more than 519 Myrtaceae species globally across 78 genera known to be susceptible to the pathogen (Giblin and Carnegie 2014; Roux et al. 2016; Soewarto et al. 2018). This successful geographical spread has spanned many countries over

several continents including Jamaica (MacLachlan 1938), U.S.A (Marlatt and Kimbrough 1979), Japan (Kawanishi et al. 2009), China (Zhuang and Wei 2011), South Africa (Roux et al. 2013), New Caledonia (Giblin 2013), Indonesia (McTaggart et al. 2016), Singapore (du Plessis et al. 2017) and more recently New Zealand (DOC 2017) (see Table 1 for a complete list).

Table 1 List of countries where *Austropuccinia psidii* has been detected as well as the year of its first detection.

Year	Country	Reference
1884	**Brazil**	Winter 1884
1884	**Paraguay**	Spegazzini 1884
1891	**Ecuador**	Stevenson 1926
1913	**Colombia**	Mayor 1913
1913	**Puerto Rico**	MacLachlan 1938
1926	**Cuba**	Seaver & Chardón 1926
1933	**Dominican Republic**	Kern et al. 1933
1934	**Venezuela**	Chardón and Toro 1934
1934	**Jamaica**	MacLachlan 1938
1945	**Dominica**	Baker & Dale 1948
1946	**Argentina**	Di Fonzo 1946
1951	**Trinidad and Tobago**	Baker & Dale 1951
1968	**Guatemala**	Schieber & Sánchez 1968
1977	**USA**	Marlatt & Kimbrough 1979
1981	**Mexico**	León-Gallegos & Cummins 1981
1987	**El Salvador**	CMI 1987
1998	**Costa Rica**	Di Stéfano et al. 1998
2002	**Uruguay**	Spegazzini 1889
2007	**Japan**	Kawanishi et al. 2009
2009	**China**	Zhuang & Wei 2011
2010	**Australia**	Carnegie et al. 2010
2013	**South Africa**	Roux et al. 2013
2013	**New Caledonia**	Giblin 2013
2015	**Indonesia**	McTaggart et al. 2016
2016	**Singapore**	du Plessis et al. 2017
2017	**New Zealand**	DOC 2017

In 2010, *A*. *psidii* was first detected in Australia, in a plant nursery on the Central Coast of New South Wales (NSW) (Carnegie et al. 2010). However, the pathway of introduction into the country remains unknown. An eradication attempt was unsuccessful (Carnegie and Cooper 2011, Carnegie and Pegg 2018) and *A*. *psidii* has since spread rapidly through eastern Australia, initially via anthropogenic activity (e.g. movement of infected plants) then more commonly via air-borne spores. Currently, the rust is present along the entire east coast of Australia, with restricted distribution in Victoria, Northern Territory and Tasmania (Agriculture Victoria 2016; DEE 2016; Westaway 2016; Berthon et al. 2018). Fortunately, it has not been detected in South Australia or Western Australia, the latter of which is a global biodiversity hotspot for plants (Brooks et al. 2002). In Australia, *A. psidii* poses a significant threat to native vegetation communities as it infects the Myrtaceae, one of the dominant plant families in Australia (Wiltshire 2004; Glen et al. 2007). Australian Myrtaceae include the iconic Eucalypts, *Melaleuca* (paperbark trees and bottlebrushes) and *Leptospermum* species. To date, there are 358 native species from 49 genera known to be susceptible to *A. psidii* in Australia through susceptibility tests and/or field observations (Giblin and Carnegie 2014; Makinson 2018). These values correspond to 19% of Myrtaceae species in Australia, but almost 57% are expected to be exposed due to overlapping climate suitability of host and pathogen, either now or in the future with climate change (Berthon et al. 2018). So far, only one *A*. *psidii* biotype (the 'pandemic' biotype) has been detected in Australia (Machado et al. 2015). Interestingly, different biotypes have been shown to infect different hosts (Coutinho et al. 1998; de Carvalho et al. 1998; Graça et al. 2013; Silva et al. 2014). As a consequence, several authors have argued that maintaining the single biotype of *A*. *psidii* in Australia – through continued biosecurity – is crucial to minimise its

ecological impacts (Carnegie and Lidbetter 2012; Carnegie and Pegg 2018; Makinson 2018).

Globally, the impacts of *A*. *psidii* on native vegetation communities are poorly understood, with only a few studies conducted in the U.S.A. (Hawaii - Loope and La Rosa 2008) and Australia (Carnegie et al. 2016, Pegg et al. 2017, 2018). The studies on impacts have focused mainly on commercial species, with a few exceptions. *Pimenta dioica* (allspice) plantations in Jamaica were devastated in the 1930s (Smith 1935; MacLachlan 1938) and outbreaks of *A*. *psidii* in *Eucalyptus* nurseries and plantations in Brazil in the 1970s were reported (Ferreira 1983; Dianese et al. 1984), resulting in significant management efforts, including clonal breeding techniques (Furtado and Marino 2003). Also in Brazil, *A*. *psidii* is a significant limiting disease in *Psidium guajava* (guava) plantations, requiring weekly fungicide application in some areas which substantially increases the management costs (Ribeiro and Pommer 2004). In the U.S.A *A*. *psidii* has caused epidemics in the invasive *Melaleuca quinquenervia* (Rayachhetry et al. 1997) and *Rhodomyrtus tomentosa* (Rayamajhi et al. 2013) in Florida, and has threatened iconic Myrtaceae (endangered *Eugenia koolauensis*) in Hawaii (Loope 2010). When *A*. *psidii* invaded Australia, it doubled the species' host range, impacting *Backhousia citriodora* (lemon myrtle) plantations resulting in increased costs to the nursery and garden industry (Carnegie and Pegg 2018) and causing severe impacts on several native species in different vegetation types (Carnegie et al. 2016, Pegg et al. 2017, 2018). The pathogen was detected in New Caledonia in 2013 adding 67 host species and 5 genera to the worldwide host species list and threatening iconic native Myrtaceae (Soewarto et al. 2018). The impacts in South Africa, Indonesia, and New Zealand are as yet unknown (McTaggart et al. 2016; Roux et al. 2016; MPI 2018).

As Australia is home to 40% of the global Myrtaceae species (2,250) and 60% of the genera (80) (Grattapaglia et al. 2012; Makinson 2014), it is vital that the ecological impacts of *A*. *psidii* are well understood. However, despite *A. psidii* being present in Australia for almost eight years, there have been few studies on its impact on native vegetation, as research has focused more on the commercial impact of the invasion rather than environmental impact (Carnegie and Pegg 2018). For example, the lemon myrtle (*Backhousia citriodora*) industry has been severely affected by *A. psidii* due to reduced leaf production and the need for fungicide application halting attempts for organic production (Doran et al. 2012; Lancaster 2017). So far, three pioneering studies have assessed the impacts of *A*. *psidii* on highly susceptible native species in natural communities within Australia (Carnegie et al. 2016; Pegg et al. 2017, 2018). Among these three, the only study that had a disease exclusion treatment (fungicide sprayed trees) was that of Carnegie et al. (2016). They assessed impacts of *A*. *psidii* at the species-level on *Rhodamnia rubescens* (brush turpentine) and *Rhodomyrtus psidioides* (native guava), both of which are now preliminarily listed as critically endangered due to *A*. *psidii* (NSW Scientific Committee 2017). Carnegie et al. (2016) reported reductions in canopy cover as well as mortality after multiple re-infection events across the range of both species. Pegg et al. (2017) assessed the community-level impacts of *A. psidii* in a Queensland wet sclerophyll forest, finding that composition of species shifted from Myrtaceae dominated to non-Myrtaceae dominated within a short time frame. In a second study they assessed the impacts of *A*. *psidii* in a coastal heathland after fire, finding that the infection of resprouting epicormic growth by *A*. *psidii* led to defoliation, dieback and mortality of highly susceptible individuals (Pegg et al. 2018). These few studies show that *A*. *psidii* is having severe impacts on several Myrtaceae species in Australia. However, the

impacts of *A. psidii* at a community-level remain poorly understood. Therefore, the overarching aim of this thesis was to investigate the impacts of *A*. *psidii* on Australian native vegetation communities.

Thesis structure and research questions

This thesis consists of six chapters: this Introduction, four data chapters which have been prepared for submission for publication in relevant scientific journals, and a Discussion which integrates the main findings. Although chapters have been prepared for different journals they have been formatted in a consistent style for this thesis. Each chapter has been prepared as a stand-alone manuscript, thus this thesis contains some unavoidable repetition of introductory material and methods.

As stated above, the overarching aim of this thesis was to investigate the impacts of *A*. *psidii* on Australian native vegetation communities. Because *A. psidii* is a relatively recent invasion, many key knowledge gaps still exist regarding its ecology and impacts. For this thesis, I identified and addressed a number of knowledge gaps that I believe were a priority. These knowledge gaps and how they were addressed is outlined briefly below.

Chapter 2: **Collating data on *A. psidii* host species, distribution and impacts**

In order to effectively monitor the spread of *A*. *psidii* and assess its impacts, we must first determine which Myrtaceous species are susceptible. The national host species list dates from 2014 and there have been limited efforts to keep it updated.

Furthermore, there is no systematic surveillance or national monitoring program to track the impact and spread of *A*. *psidii* in Australia.

To address this, *Chapter 2* describes the outcomes from a questionnaire designed to gather knowledge on the host species, distribution and impacts (on specific plant tissues) of *A. psidii* in Australia (published in *Biological Invasions*). More specifically, we aimed to identify species susceptible to *A*. *psidii* (which had not yet been identified as host species in Australia) and the severity of infection in these species. In addition, we aimed to determine the current distribution of *A*. *psidii* in Australia as well as the impacts it is having and the control measures, if any, that are currently used to moderate these impacts. More than 500 questionnaires were distributed to employees of botanic gardens, national parks, local councils, nursery and forestry industries, in all states where *A*. *psidii* is known to be present (New South Wales, Queensland, Northern Territory, Victoria and Tasmania). We received more than 250 responses that provided invaluable up-to-date information on the host species, distribution and impacts of *A*. *psidii*, which will assist in making management decisions relating to this pathogen across Australia.

Chapter 3: ***Austropuccinia psidii*** **susceptibility tests**

The aim of *Chapter 3* was to extend on *Chapter 2* and test the susceptibility to *A*. *psidii* of a range of previously untested Myrtaceous species/sub-species. To do this, seedlings from 24 species/sub-species were grown from seed under controlled conditions in glasshouses, and then inoculated with *A*. *psidii*. The tested species included ones that are (1) listed as endangered under national or state-level legislation and have a large overlap with *A*. *psidii* climatically suitable areas in

eastern Australia, (2) not endangered but have a significant overlap with *A. psidii* climatically-suitable areas, (3) previously identified as a host species (*Chapter 2*) but need confirmation in order to be included in the national host species list, and 4) within a previously untested genus. Different sub-species and provenances were also tested to assess for intra-specific variation. This chapter is in review at *Australasian Plant Pathology*.

Chapter 4: **Impacts of *A. psidii* on susceptible species after fire**

Fire is a natural component in the ecology of Australian landscapes and plays an important role in shaping the structure and function of many native plant communities. As a result, a large proportion of Myrtaceous species in Australia have co-evolved to resprout after this common disturbance. These resprouting species (assuming they are susceptible) are particularly vulnerable to *A. psidii* infection after fire as they produce large amounts of new growth in a short time period when there is relatively little other photosynthetically-active foliage present on the plant.

Although the interaction between fire and *A. psidii* was identified as a potential threat to native Myrtaceae species prior to *A. psidii* introduction into Australia (Grgurinovic et al. 2006), to date only one study has attempted to test these impacts (Pegg et al. 2018).

Therefore, the aim of *Chapter 4* was to address this knowledge gap by testing the impacts of *A. psidii* on eight resprouting susceptible host species after fire in a controlled glasshouse experiment. We hypothesized that resprouting plants inoculated with *A. psidii* would show differences in 1) plant architecture, with

reductions in height and increases in stem number compared to control plants; 2) growth, with reduced leaf biomass (due mainly to defoliation), and 3) biomass allocation, with reductions in specific leaf area (due to increased leaf distortion) and leaf mass fraction (due to both defoliation and resource diversion to defence) compared to control plants. This chapter has been prepared for submission to *Oecologia.*

Chapter 5: **Impacts of *A. psidii* at the community-level**

Although a handful of studies have shown the detrimental impacts of *A*. *psidii* in the field at the species-level (discussed above; Carnegie et al. 2016; Pegg et al. 2017, 2018), the potential flow-on community-level impacts are yet to be assessed. Therefore, the aim of *Chapter 5* was to assess the community-level impacts of *A*. *psidii* as a result of infection of two highly susceptible rainforest mid-canopy species, *Rhodamnia rubescens* and *Rhodomyrtus psidioides*. This chapter describes a two year large-scale disease exclusion (fungicide spray application) field experiment across six rainforest sites. The community-level impacts assessed were changes in the richness and abundance of the co-occurring understorey species and seedling recruitment of the study species.

We hypothesized that *A*. *psidii* infection would result in increased defoliation and mortality (both resulting in increased canopy transparency) of *R*. *rubescens* trees. We predicted that because of the increased light availability, there would be an increase in the richness and abundance of co-occurring understorey species. We also predicted that *A*. *psidii* infection would directly reduce the recruitment of *R*. *rubescens* and *R*. *psidioides* seedlings in the understorey, resulting in increases in the

richness and abundance of co-occurring understorey species. This chapter has been prepared for submission to the *Journal of Applied Ecology*.

Chapter 6: **Discussion**

In this chapter I summarise the key findings of my thesis as well as discuss the contribution of these findings to the broader literature. I then suggest ongoing and future *A. psidii* management options and avenues for future research.

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CHAPTER TWO

Myrtle rust on the move: New insights to the distribution and hosts of *Austropuccinia psidii* in Australia

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Abstract

Austropuccinia psidii is a fungus native to South and Central America that affects the growth and reproduction of species in the Myrtaceae family. *Austropuccinia psidii* causes a disease known as myrtle rust, and was first detected in Australia eight years ago in New South Wales. Since then it has spread rapidly along the east coast, and to date it is known to infect more than 358 native Myrtaceae species in Australia. Despite this, its rapid spread is not well documented and the potential threat to other Myrtaceae species remains unknown, as there is no systematic surveillance or monitoring program in Australia. In order to better understand *A. psidii*'s geographic extent and impacts on Australian landscapes, a survey was sent to national park, botanical garden, council, nursery and forestry agency employees in all states where the disease is known to be present. More than 500 surveys were sent, and 254 responses were received. The survey confirms that *A. psidii* is widespread in New South Wales and Queensland urban environments as well as in native vegetation communities. Four new host species were confirmed, as well as four new local government areas in two different states reporting *A. psidii* infection. Severity of infection was classified as medium to high for most susceptible species, with especially negative impacts for *Rhodamnia rubescens* and *Rhodomyrtus psidioides.* These survey results provide invaluable up-to-date information on the geographical distribution, host species and impacts of *A. psidii*, which will assist in making management decisions relating to this pathogen across Australia.

Keywords: Puccinia psidii; Invasive species; Plant pathogen; Myrtaceae

Introduction

Invasive plant pathogens represent a significant biodiversity conservation challenge worldwide, with impacts ranging from the species- to ecosystem-level. Well known examples include the exotic fungus *Cryphonectria parasitica*, the cause of chestnut blight (Anagnostakis 1987), which has caused significant mortality of the American chestnuts (*Castanea dentata*); and the soil-borne pathogen *Phytophthora cinnamomi*, which has not only altered the structure of *Eucalyptus* forests in Western Australia (WA) but also affected faunal species in NSW and Victoria that rely on affected forests for habitat [e.g. *Pseudomys fumeus* (smoky mouse), *Isoodon obesulus obesulus* (southern brown bandicoot) (Cahill et al. 2008)]. Pathogens can be particularly destructive when they infect keystone species, as well as endemic, endangered or rare species, given their intrinsic value for biodiversity conservation (Ellison et al. 2005; Loo 2009).

Austropuccinia psidii is a pathogenic fungus native to South and Central America that affects species in the Myrtaceae family (Coutinho et al. 1998; Glen et al. 2007). It causes a disease known as myrtle, eucalyptus, guava or ohia rust, depending on the species or group of species that it affects in different countries. Myrtaceae is the dominant plant family in Australia (Wiltshire 2004), which is why the introduction and spread of *A. psidii* is of particular concern for Australian vegetation, including the iconic *Eucalyptus*, *Melaleuca* and *Leptospermum* species. *Austropuccinia psidii* infects young growing tissues such as new leaves, tender shoots, flowers and fruits (Coutinho et al. 1998; Glen et al. 2007). Responses to infection are species specific, with symptoms ranging from purple flecks in resistant species to characteristic yellow urediniospores that can lead to leaf distortion, defoliation,

reduced reproduction (by infection of flowers and fruits) and death (Rayachhetry et al. 2001; Uchida et al. 2006; Carnegie et al. 2016). Carnegie et al. (2016) assessed the impacts of *A. psidii* on two highly susceptible native species in Australia, brush turpentine (*Rhodamnia rubescens*) and native guava (*Rhodomyrtus psidioides*). In these species, as a result of *A. psidii* infection, the canopy cover was significantly reduced by 76% and 95% respectively, with repeated damage causing mortality in 12% and 57% of these two species across their native range (Carnegie et al. 2016). Pegg et al. (2017) also reported significant levels of canopy damage and tree mortality in a range of Myrtaceae species in moist rainforest ecosystems unique to Australia. It is clear that *A. psidii* is having severe impacts at the species-level, which may have potential consequences at the ecosystem-level.

The distribution of *A. psidii* is dynamic with spores being transported long distances by wind and animals allowing it to spread easily (Uchida et al. 2006). The pathogen has spread to several countries and continents including North America (Florida and Hawaii), Southeast Asia (Japan, China, Indonesia and Signapore), South Africa, New Caledonia and more recently New Zealand (Marlatt and Kimbrough 1979; Kawanishi et al. 2009; Roux et al. 2013; Zhuang and Wei 2011; DOC 2017; Soewarto et al. 2018). It was first detected in Australia north of Sydney, New South Wales (NSW) in 2010 (Carnegie et al. 2010). The rust is currently present along the east coast of Australia with currently restricted distribution in Victoria, the Tiwi Islands, Northern Territory (NT) and Tasmania (TAS) (Berthon et al. 2018), a distance of over 5000 km. The most recent expansion in distribution reported is Lord Howe Island, NSW (Kelly 2016). At present the only states where it has not been reported are South Australia (SA) and Western Australia (WA).

In order to effectively monitor the spread of *A. psidii* and assess its impacts, we must first determine which Myrtaceous species are susceptible. As determined by laboratory infection tests and field observations, at least 358 Australian Myrtaceous species are known to be susceptible to *A. psidii* (Giblin and Carnegie 2014; Makinson 2018). However, the national host species list dates from 2014 and there have been limited efforts to keep it updated. There is no systematic surveillance or national program of monitoring the spread and impact of *A. psidii* in Australia. Up-to-date information on the distribution and impacts of *A. psidii* is essential for governments, scientists and natural resource managers to make effective decisions for what quarantine, management and control measures should be taken. The aim of this study was to collate information on the effects of *A. psidii* in Australia. More specifically, we aimed to identify species susceptible to *A. psidii* (which are not yet identified as host species in Australia) and the severity of infection in these species. In addition, our objective was to determine the current distribution of *A. psidii* in Australia as well as the impacts it is having and the control measures, if any, currently used to moderate these impacts.

Methods

We created an online survey in order to gather and compile the currently fragmented information concerning *A. psidii* distribution, host species and impacts in Australia (See Appendix A1 for the complete survey). All research was conducted with the approval of the Faculty of Science and Engineering Human Research Ethics Sub-Committee at Macquarie University (Reference number: 5201500739, Appendix A2). The software 'Qualtrics' (https://www.qualtrics.com; Macquarie University) was

used to support the survey. An internet link was created and sent to participants via email to be answered online. The completion of the questionnaire was taken as indication of voluntary consent to participate.

The survey was sent to employees from a number of informed organisations including botanical gardens, national parks, local land services, councils, forestry agencies, nurseries and bush regeneration groups in all states where *A. psidii* is present in Australia [NT, Queensland (QLD), NSW, Victoria (VIC) and TAS]. The email accounts were obtained from official internet websites or via personal recommendation. The survey was sent in December 2015 to 548 email addresses and from here some contacts re-distributed the survey or published the link in professional forums.

The questionnaire comprised of a series of yes / no questions with free responses following 'yes' answers, as well as adequate space for other comments. Participants were asked to identify any species infected by *A. psidii* they had seen, their location [locality name and local government area (LGA)] and the plant community type they occurred in. The list of LGAs which were reported to contain *A. psidii* was collated and then mapped using the spatial analysis software ArcMap 10.3 (ESRI 2012). These were then compared with an updated dataset of myrtle rust records (Berthon et al. 2018) to find LGAs which had not previously been reported to contain *A. psidii*. The separation of plant community type into natural (rainforest, tall open forest, coastal woodland, riparian / gully, swamp sclerophyll forest, dry sclerophyll forest, and closed forest) and human mediated landscapes (garden, street tree, plant nursery, botanical garden, and park) was made *a posteriori* for analysis purposes. For this category participants used a variety of terminologies, which were

grouped together if similar or synonymous (i.e. tall open forest with wet sclerophyll forest and ornamental with garden; for complete list refer to Appendix A3).

Information was also obtained on which plant tissues were affected (e.g. leaves, flowers, fruits), level of infection (low, medium or high; see Table A4 for more details), presence of defoliation and mortality. Further questions referring to control measures taken such as the cutting of infected branches, removal of infected individuals or fungicide application; as well as the respondent's personal opinion regarding the possibility of *A. psidii* being a long-term threat for native communities were also asked. If participants said they had not seen *A. psidii* in their area, they would skip the following questions and be provided with a short explanation about the pathogen and a link to the national host species list (https://www.anbg.gov.au/anpc/images/Puccinia%20psidii%20Australia%20Hostlist%2024 Sept2014%20WORDtable.pdf). Finally, the survey was concluded by asking all participants whether they had received information from the government about this *A. psidii* invasion, who they were employed by (local, state or federal government or private consulting) and if they were willing to receive the results of the survey as well as updated information on the invasion of *A. psidii*.

The survey was closed on February 2016, having 254 responses, from which 200 participants replied to all the questions. As not all the questions were compulsory, there were differences in sample sizes across the different components. Most answers were descriptive, giving qualitative results, therefore no statistical analyses were undertaken.

Results & Discussion

New host species found for Austropuccinia psidii *in Australia*

Respondents of the survey identified 51 susceptible (infected) species from 29 genera, including five species not present in the updated national list of host species for *A. psidii* (Table 1) [see Giblin and Carnegie (2004) for original host national species list, updated by Makinson (2018)]. Species that dominated the list of recorded susceptible species were the natives *Melaleuca quinquenervia*, *Rhodamnia rubescens*, *Rhodomyrtus psidioides* and the exotic *Syzygium jambos*. The new species reported were *Lophomyrtus obcordata* in VIC and *Kunzea parvifolia*, *Leptospermum myrtifolium*, *Eucalyptus amplifolia* and *Lophostemon confertus* in NSW.

Table 1 Plant species or genera reported as infected with *A. psidii* in Australia, number (N) of respondents that have seen these taxa with symptoms of infection or pustules, and species name synonyms. In total 51 species were mentioned in 29 different genera. Previously unknown susceptible species are shown in bold. If not native, exotic origin is specified between brackets.

Species name / Genus	Synonyms	N
Acmena spp.		1
Acmena smithii	*Syzygium smithii*	2
Acmenospermum claviflorum	*Syzygium claviflorum*	1
Agonis spp.		3
Agonis flexuosa "afterdark"		1
Agonis flexuosa "nana"		2
Austromyrtus spp.		5
Austromyrtus dulcis		4
Backhousia myrtifolia (Cinnamon myrtle)		3
Backhousia oligantha		1
Callistemon viminalis		1
Chamelaucium uncinatum (Geraldton wax)		2
Chamelaucium hybrids		1
Corymbia maculata		1
Decaspermum humile		2
Eucalyptus amplifolia (Cabbage gum)		1
Eucalyptus tereticornins		1

Eugenia reinwardtiana		3
Gossia spp.		3
Gossia acmeniodes		1
Gossia floribunda		1
Gossia fragrantissima		1
Gossia inophloia	*Austromyrtus inophloia*	1
Kunzea parvifolia		1
Lenwebbia lasioclada		1
Leptospermum laevigatum		1
Leptospermum myrtifolium		1
Lithomyrtus obtusa		1
Lophomyrtus obcordata		1
Melaleuca spp.		3
Melaleuca leucadendra		2
Melaleuca nodosa		1
Melaleuca quinquenervia		13
Metrosideros spp.		1
Metrosideros kermadecensis (Exotic, New Zealand)		1
Myrtaceae family/species, myrtles		5
Myrtus communis		2
Rhodamnia spp.		9
Rhodamnia acuminata		1
Rhodamnia maideniana		1
Rhodamnia rubescens	*Rhodamnia trinervia*	27
Rhodamnia spongiosa		1
Rhodomyrtus spp.		2
Rhodomyrtus pervagata		1
Rhodomyrtus psidiodes		10
Rhodomyrtus sericea		1
Rhodomyrtus tomentosa (Exotic, Asia)		1
Sphaerantia discolor		1
Syncarpia glomulifera		4
Syzygium spp.		4
Syzygium anisatum	*Anetholea / B. anisata*	1
Syzygium australe		1
Syzygium corynanthum		1
Syzygium dansiei		1
Syzygium jambos (Rose apple; Exotic, Asia)		12
Syzygium luehmannii		1
Syzygium paniculatum		1
Syzygium smithii var. minor		1
Syzygium tierneyanum		1
Tristaniopsis spp.		1
Tristaniopsis laurina		2
Uromyrtus metrosideros		1
Waterhousea spp.		1
Xanthostemon fruticosus (Red Chief)		1

Signs of infection were confirmed by the Curator Environmental Horticulture, Peter Symes (Royal Botanical Gardens, VIC), for *Lophomyrtus obcordata,* which has been included in the national host species list by the *Myrtle rust and environment impacts working group*. *Lophomyrtus obcordata* is not a native species in Australia, but endemic to New Zealand. *Kunzea parvifolia* and *L. myrtifolium* were confirmed by the Manager of Eurobodalla Regional Botanic Gardens, who observed symptoms on new leaves and on some stems, with low to medium levels of infection. *Eucalyptus amplifolia* was reported to have infected leaves and mortality in a nursery. However, this could not be supported with photographic proof. The species has previously been shown to be susceptible in Japan (Kawanishi et al. 2009), and *E. amplifolia* subspecies *amplifolia* was found to be susceptible in Brazil by Zauza et al. (2010). Even though it was known to be susceptible in other countries, this is the first time it has been reported as a host in Australia, and as such will be added to the National Host species list. We performed additional glasshouse susceptibility tests on *E. amplifolia* subspecies *sessiliflora* (Blakely) and confirmed it as host as well (*Chapter 3*). Finally, it was reported that *L. confertus* showed slight symptoms mainly on new growth where leaf tips tended to die, however previous tests by Sandhu and Park (2013) found it to be resistant, and it had not been reported previously from surveys in NSW (Carnegie and Lidbetter 2012) or Qld (Pegg et al. 2014). Thus, we could not confirm *L. confertus* as a host of *A. psidii*.

Following on from these susceptibility assessment outcomes, it is important that the impacts of *A. psidii* on these new host species are assessed. If they are found to be highly susceptible, seed collection should be encouraged for species conservation purposes (i.e. establishment of a seed bank).

Severity of infection

Of the 23 species that severity of infection information was obtained for, it was reported that 13 species had high severity while a further seven species had medium severity of infection. *Rhodamnia rubescens*, *R. psidioides* and *Gossia inophloia* (before *Austromyrtus inophloia*) were reported to have particularly high severity of infection leading to mortality (Table 2). Other species recorded as suffering mortality were *Metrosideros kermadecensis* (medium susceptibility), *Rhodomyrtus tomentosa* and seedlings of *E. amplifolia* and *E. tereticornis*. Most species were described as showing symptoms of infection on newly developed leaves (39 of 51 species), while *Melaleuca quinquenervia* and *Leptospermum myrtifolium* had infected stems as well. *Decaspermum humile*, *Eugenia reinwardtiana*, *Rhodamnia maideniana*, *R. rubescens* and *Syzygium paniculatum* were reported to have infection on flower buds and/or fruits, which may reduce their reproductive output. Interestingly in terms of synergetic effects of pest interactions, a participant found that *Syzygium* cultivars become more severely infected with *A. psidii* after psyllid attack. This may be due to new leaf and shoot production resulting from psyllid attack being especially susceptible to *A. psidii*. In Florida, USA, where *M. quinquenervia* is regarded as a weed, infection by *A. psidii* is compounded by attack from the weevil *Oxyops vitiosa* (Rajamajhi et al. 2010). Similarly, Pegg et al. (2018) reported the combined impacts of repeated infection by *A. psidii* and a range of insect damage types, including mirids, on *M. quinquenervia* in northern NSW.

Table 2 List of Myrtaceae species susceptible to *A. psidii* with information on severity of infection, tissues affected, occurrence of defoliation and mortality (Y = yes, N = no). Crucial information (i.e. when susceptible tissues are flowers or fruits and occurrence of mortality) can be found in bold. New information not reported before is underlined.

Species	Severity	Tissues	Defoliation	Mortality
Acmena smithii	Medium	Leaves, new growth		
Acmenospermum claviflorum		Leaves		
Agonis flexuosa "nana"	High	Leaves, all growth		N
Austromyrtus dulcis	Med to high	Shoot tips, leaves	Y	
Austromyrtus inophloia	High	Leaves		**Y high**
Austromyrtus spp.	Med to high	Leaves, stems	Y	N
Backhousia myrtifolia	High	Young tender foliage		
Backhousia oligantha		Leaves		
Chamelaucium hybrids	Medium	Leaves, **flower buds**		
Chamelaucium uncinatum		Leaves		
Corymbia maculate	Low to med	Leaves		N
Decaspermum humile	Medium	L, **flowers, fruits**		
Eucalyptus amplifolia		Leaves		**Y**
Eucalyptus tereticornis		Leaves		**Y**
Eugenia reinwardtiana	High	Leaves, **fruits**	Y	
Gossia floribunda	Med to high	Leaves	Y severe	
Gossia fragrantissima	Low	New leaf tips		
Kunzea parvifolia	Medium	New shoots		
Lenwebbia lasioclada		Leaves		
Leptospermum myrtifolium	Low to med	Leaves, stems		
Lophomyrtus obcordata		New shoots & **fruits**		
Melaleuca leucadendra		New growth		
Melaleuca quinquenervia	Low to high	Leaves, green stems		
Metrosideros kermadecensis		Leaves		**Y** med
Rhodamnia acuminate		Leaves		
Rhodamnia spp.	Medi to High	Leaves, stems	Y	Y
Rhodamnia maideniana	Med to high	Leaves and **fruits**		
Rhodamnia rubescens	High	L, **flowers, fruits**	Y severe	**Y high**
Rhodamnia spongiosa	High	Leaves		N
Rhodomyrtus pervagata		Leaves		
Rhodomyrtus psidioides	High	Leaves, all parts	Y	**Y high**
Rhodomyrtus tomentose				**Y**
Sphaerantia discolour		Leaves		
Syncarpia glomulifera	Medium	Leaves		
Syzygium anisatum	High	Young tender foliage		
Syzygium corynanthum		Leaves	Y	
Syzygium dansiei		Leaves	Y	
Syzygium jambos	Low to high	Leaves only	Y	N
Syzygium luehmannii	Low	Leaves	Y	

Syzygium paniculatum	Low	Leaves, **fruits**	Y
Syzygium tierneyanum		Leaves	Y
Tristaniopsis laurina	Low	Leaves	
Uromyrtus metrosideros		Leaves	

This study has collated information on which tissues are affected by *A. psidii*, as well as severity of infection, defoliation and mortality at a species-level, for several Myrtaceae species not previously reported by any other study, including Pegg et al. (2018) (see Table 2, where underlined information is new to the bibliography). The infection of flowers and fruits in species including *D. humile*, *E. reinwardtiana*, *R. maideniana*, *R. rubescens* and *S. paniculatum*, may reduce the reproductive fitness of the species and subsequently have long-term population-level effects. This information is critical in assessing the vulnerability of susceptible species to *A. psidii* so that informed management decisions can be made to ensure the conservation of species that are most at risk.

Expanded distribution of Austropuccinia psidii *in Australia*

Four new local government areas (LGAs) where myrtle rust has not been detected before were reported in this survey: two in QLD (Burdekin and Gladstone) and two in NSW (Singleton and Muswellbrook) (Fig. 1 and Table A5). Note that if respondents did not report myrtle rust in a particular area, it does not necessarily mean that it is absent from that area.

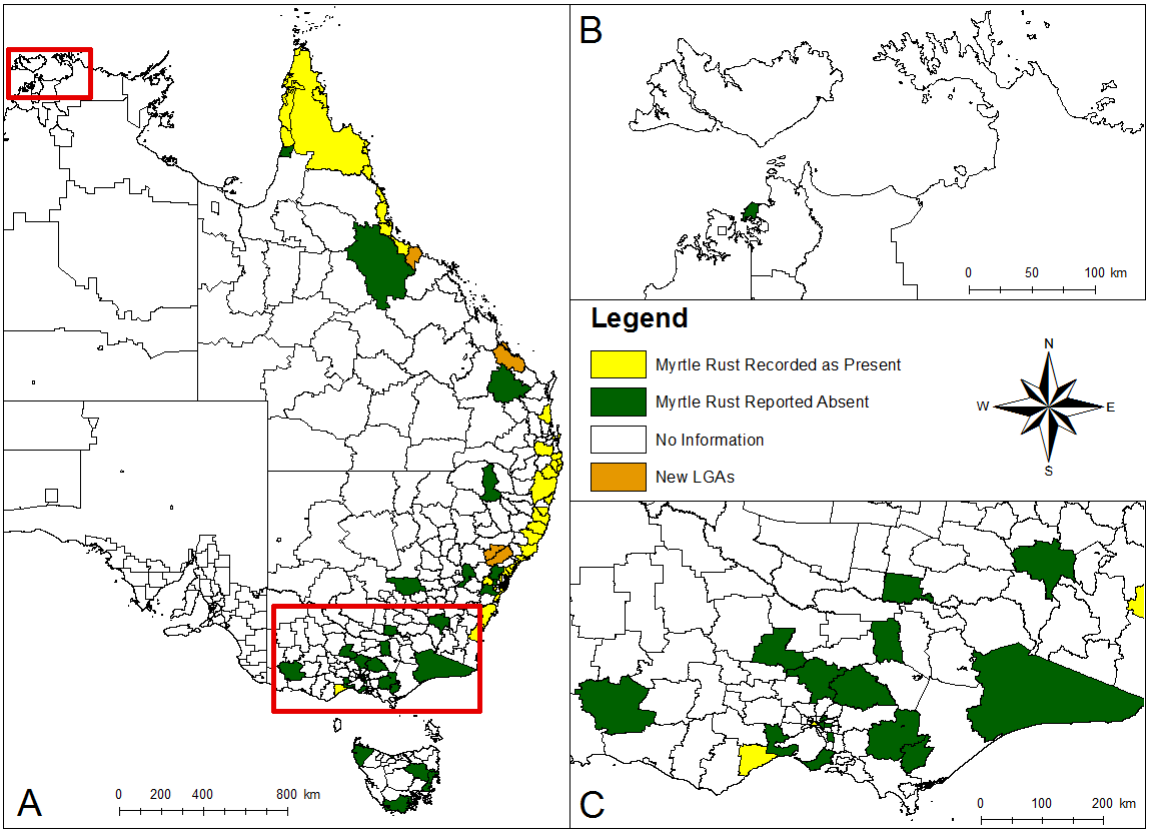

Fig. 1 (A) Local government areas (LGAs) where *A. psidii* was observed ("Present", in yellow) and where it has not been detected by participants ("Not present", in green). LGAs where we do not have information were left uncoloured and new LGAs can be found in orange. Zoomed in maps of the Northern Territory **(B)** and Victoria **(C)** help visualize different LGAs.

Given that the pathogen continues to increase in its distribution, containment measures are still needed to avoid *A. psidii* from spreading into South Australia and into Western Australia, the latter of which contains a high number of endemic species and is a recognised global biodiversity hotspot. Given its easy spread through airborne spores, *A. psidii* has recently arrived in New Zealand (DOC 2017), and unless it encounters climate barriers (e.g. dry conditions, low/high temperatures; see Kriticos et al. 2013; Berthon et al. 2018) it seems likely that it will spread to the two remaining unaffected Australian states. In a more international context, quarantine is

crucial to prevent new strains of the pathogen from arriving into the country that could lead to an increase in the number of susceptible host species or in the level of infection of those already vulnerable. This could be triggered by the new strain itself, or by hybridization of strains, as there are currently at least nine biotypes of *A*. *psidii* that have been identified worldwide (Stewart et al. 2018). Restrictions to Myrtaceae species imports are currently underway for all seeds arriving from *A*. *psidii* host countries. Imports require a phytosanitary certificate stating them to be free of quarantine pests (BICON 2018). Considering these reasons, we suggest that it is imperative that a nationally coordinated strategy provides the public and natural resource managers in particular, with information on *A. psidii* to enable early detection and containment. The fact that this information distribution currently is not happening, at least according to participants of this survey in the states were *A*. *psidii* is known to be present, is alarming, as the lack of awareness of land managers and stakeholders to the threat will make it difficult to maintain appropriate quarantine and containment measures. South Australia, for example, has restrictions on the import of Myrtaceae plants from states in which *A*. *psidii* has been detected, with material needed to be certified in order to enter the state, including nursery stock, cut flowers, fruit, germplasm, seed and tissue culture (PIR 2018).

Austropuccinia psidii was reported in a wide range of landscapes in NSW and QLD, ranging from urban areas to native environments. In contrast, the fungus was reported only in urban areas in VIC while no occurrences were reported for NT or TAS in our survey, although the rust is present (Table 3). Overall, slightly more respondents reported an absence of the rust in their area (51.6%) compared to those which reported that they had seen it (48.4%).

Table 3 Number of *A. psidii* detections (or lack of them) by stakeholders per state in Australia (given that some questions were not answered, total numbers differ).

State	Yes	No
	123	**131**
NSW	89	44
QLD	15	7
VIC	5	35
TAS	0	12
NT	0	4
ACT	0	5

In natural landscapes, *A. psidii* was most frequently observed in rainforests (51%, N = 90) and sclerophyll forests (24%, N = 90) (Fig. 2a). For urban landscapes, private gardens (53%, N=72) as well as street trees and nurseries (17% each, N=72) were most frequently observed to have *A. psidii* infection (Fig. 2b). This may be due to humidity being higher in rainforest and private gardens (naturally in the former and artificially due to frequent watering in the second) which creates optimal conditions for *A. psidii* spores to germinate (MacLachlan 1936; Ruiz et al. 1989). Frequent watering in gardens also favours host growth which means increased density of susceptible new tissues. It is also possible that the rust is more common in gardens or other populated areas due to facilitated spore dispersal by humans. It should be noted that urban landscapes may be over-represented in the survey as respondents generally spend more time in these landscapes and there is a greater density of respondents based on natural resource management units.

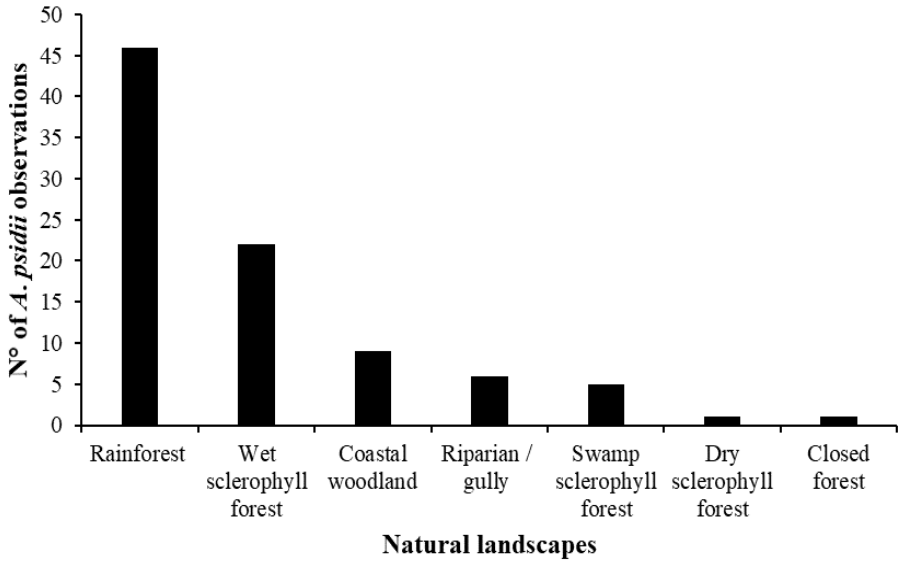

Fig 2a The natural plant community types where respondents recorded occurrence of *Austropuccinia psidii*.

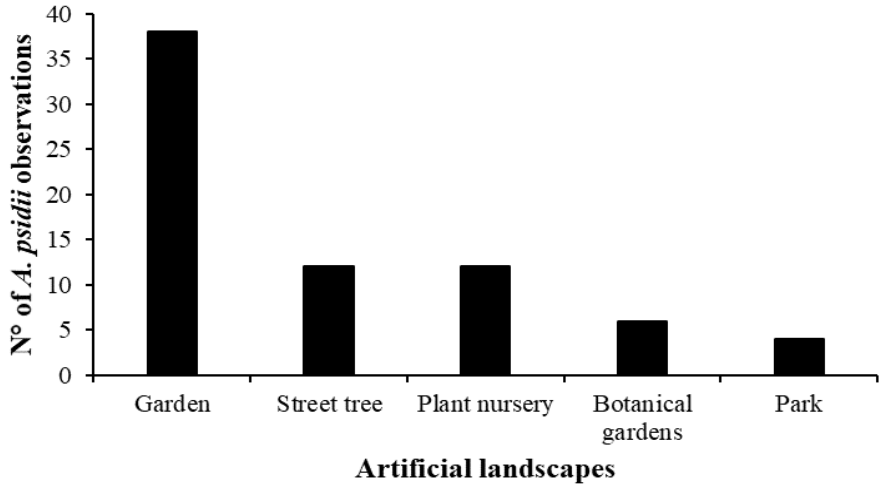

Fig 2b The human mediated environments where respondents recorded occurrence of *Austropuccinia psidii*.

The majority (91.46%) of participants surveyed that reported the occurrence of *A. psidii* (48.4%) were willing to be contacted about the threat and receive more information. People who did not report seeing the rust or did not know what it is

(51.6%), were less likely to be interested in being contacted (83.18%). A large proportion of the people surveyed work for the government (76%), mostly at the local level (77% of government workers) followed by state (19% of government workers) and then federal (4% of government workers) level. The remaining respondents work either for non-profit organisations (12%), private consulting companies (9%) or forestry agencies (3%).

Control methods dependant on time since arrival and jurisdiction

More than half of the respondents (60%, N=47) that reported *A. psidii* do not attempt to control or contain it. Given that *A. psidii* is now widespread in many areas, fungicide treatment does not seem to be an appropriate control measure. The exception to this is VIC, where the arrival of *A. psidii* is more recent, and three out of four respondents (75%, N=4) are trying to control or contain it, by either fungicide application or removal and burying of infected individuals. In general, the lack of control refers mostly (78%) to infected natural landscapes (33, N=42). Reasons given by respondents for not taking action, especially in NSW and QLD, were 'control being fruitless', 'not feasible' or the area not included in their jurisdiction. Other reasons mentioned were time constraints, and control measures being onerous or expensive.

When control measures were applied, several different methods were used. These ranged from cutting infected branches or removing infected shrubs/trees, to fungicide spray. Fungicide application was mainly undertaken by nurseries and botanical gardens using the following products: mancozeb, triforine, copper oxychloride and copper sulphate.

Long term threat

Most stakeholders (53%) surveyed think that *A. psidii* undoubtly represents a long-term threat to specific species and/or plant communities in Australia. A further 14% think that there is a possibility it may be a long term threat, 16% think it is no threat while 17% are not sure (N = 76). Stakeholders who believe *A. psidii* is a threat argued that it is likely to have devastating effects on the composition and structure of affected plant communities. It was also suggested that the current single strain of *A. psidii* may mutate or recombine into multiple new strains both within Australian and overseas. To a lesser extent, stakeholders expressed a concern of infection of new foliage post-fire, as this disturbance is common in Australian communities and *A. psidii* infects new growth. This may significantly affect post-fire dynamics and vegetation composition in fire-prone communities.

Impacts on native plant communities are still being assessed, but pioneer studies are showing significant negative impacts (Carnegie et al. 2016; Pegg et al. 2017). A long term threat has already been declared for *Rhodamnia rubescens* (brush turpentine) and *Rhodomyrtus psidioides* (native guava), two highly susceptible species which have been preliminary listed as *critically endangered* as they may become extinct in the near future due to *A. psidii* infection (Carnegie et al. 2016; NSW Scientific Committee 2017).

Participants who believed that *A. psidii* is not a long term threat cited that the impacts of *A. psidii* do not seem to be as severe as those initially predicted when the rust first arrived in Australia. We suggest that this opinion should be taken cautiously, as impacts on species abundance and vegetation composition may take a

long time to be realised, and the precautionary principle suggests that quarantine, containment and management of *A. psidii* should be a priority. Evenmore, while the greatest impact was expected to be on regeneration, personal observations by A. Carnegie and G. Pegg highlighted the speed and level of decline in mature trees, some exceeding 25m in height.

Changes in species selection for street landscapes due to A. psidii

As a result of *A. psidii*, a number of botanical gardens and nurseries no longer cultivate highly susceptible Myrtaceae species. For example, the highly susceptible *Austromyrtus inophloia* (Blushing Beauty) is no longer grown in nurseries and *Uromyrtus metrosideros* has been removed from Joseph Banks Native Plants Reserve (NSW). Furthermore, there are examples where local governments no longer recommend the use of specific species in urban landscapes as is the case with *A. flexuosa, Eugenia reinwardtiana, M*. *quinquenervia*, *R. rubescens* and *S*. *jambos*. Updated information on species susceptibility should be made widely available to the nursery and landscape architecture industries and authorities responsible for planting and management of street trees, parks and gardens. In this regard, the project developed by Pegg et al. (2018) made publicly available information on species susceptibility through the Plant Biosecurity Cooperative Research Centre (PBCRC) website (https://www.pbcrc.com.au/publications/pbcrc2206). It should be mentioned that all state biosecurity websites have information on *A*. *psidii* and host species lists, although often these have not been updated for several years.

Provision of information by government agencies

The majority of participants (80%, N=73) reported they had received information from a government agency, mainly in NSW (73%), but also in QLD (20%) and VIC (7%, N=59). According to the respondents, in the first years of *A. psidii* detection and spread in Australia (between 2010 and 2012) government agencies such as NSW Department of Primary Industries **(**DPI**),** and the equivalents in QLD (Biosecurity QLD, Department of Agriculture and Fisheries) and VIC (DEDJTR, Department of Economic Development, Jobs, Transport and Resources), distributed information on how to identify, contain and prevent *A. psidii* spread.

While initially 80% of participants received information about *A. psidii* from government agencies, 30% declared that at present they no longer receive information. Three respondents commented that the *A. psidii* invasion was badly handled by biosecurity as well as some state agencies leading to a poor outcome. For example, one stakeholder complained that funds had been allocated for genetic studies instead of containment when the rust was declared no longer technically feasible to eradicate by the Consultative Committee on Emergency Plant Pests (CCEPP).

A nationally coordinated group is needed in Australia in order to create and keep an updated open access database with all current information available on *A. psidii*. Progress has been made with the initiation of the *Myrtle rust and environment impacts working group* – a group of independent, unfunded, researchers who recognise the potential threat of *A. psidii* to Australian vegetation. Respondents that have seen *A. psidii* expressed their willingness to be contacted again about this issue

and receive more information, which can be interpreted as good prospects for national collaboration.

Conclusions

In this study we collated information from a wide range of respondents on the invasion of *Austropuccinia psidii* in Australia. We identified four confirmed new host species in Australia, three native (*Eucalyptus amplifolia*, *Kunzea parvifolia*, *Leptospermum myrtifolium*), and one exotic (*Lophomyrtus* obcordata, New Zealand), thus increasing the number of native susceptible species to 361 in 49 genera (Giblin and Carnegie 2014; Makinson 2018, *Chapter 3*). The identification of four new locations in QLD and NSW suggests that this pathogen is still increasing its distribution and highlights the current lack of systematic surveillance and reporting of *A. psidii* in Australia to track these changes. We recommend that the Australian government reinstate information distribution to land managers and the public as this study shows they are willing to receive it and have high concern for the potential impact of the pathogen. Furthermore, we support continued management directions currently in place which focus on quarantine measures to avoid the rust spreading to SA and WA as well as maintaining biosecurity restrictions to stop different strains from entering Australia.

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CHAPTER THREE

Endangered species face an extra threat: Susceptibility to the invasive pathogen *Austropuccinia psidii* (myrtle rust) within Australia

This chapter is in review at *Australasian Plant Pathology.*

My contribution to the research and paper: Concept – 70%; Data collection – 70%; Analysis – 60%; Writing – 70%

Abstract

Austropuccinia psidii (myrtle rust) is an invasive fungus native to South America that infects young growing tissues of species in the Myrtaceae family, one of the dominant plant families in Australia. It was first detected in Australia in 2010 and has since spread along the east coast, with dispersal facilitated by windborne spores. To date 360 native species from 49 genera have been found to be susceptible, but the vast majority remain untested (81%). The aim of this study was to test a range of species whose susceptibility status remains unknown, including endangered species, species with a large distribution overlap with *A*. *psidii* and species from a genus that has not been tested before. Different sub-species and provenances were also tested to assess for intra-specific differences. Seedlings were grown in glasshouses from seed and then inoculated with *A. psidii*. Of the 24 tested species/sub-species, 18 (12 endangered) were susceptible to *A*. *psidii* to varying degrees (including the new genus *Triplarina*), while one presented low susceptibility and six were resistant. The most susceptible species were the critically endangered *Callistemon megalongensis* (synonym *Melaleuca megalongensis*), and the endangered *Eucalyptus copulans*, *E*. *parvula*, *E*. *scoparia* and *Melaleuca irbyana*. No significant differences in susceptibility were found between sub-species or provenances. From these results we suggest that the susceptible species we have identified will be vulnerable to infection in their native ranges in the future, if they have not already become infected. For particularly susceptible species, *A*. *psidii* should be considered as a major additional threat and therefore appropriate control measures need to be incorporated into existing threatened species plans. Monitoring of susceptible species' populations in the wild and seed collection for seed banking are the vital first steps in ensuring their conservation.

Keywords: Exotic – Fungus – Inoculation trials - Myrtaceae - Native communities - *Puccinia*

Introduction

Of the five species of pathogenic rust fungi (Order: Pucciniales) that are known to infect plants in the Myrtaceae family (Hennen et al. 2005; Simpson et al. 2006), none have been as devastating in their impact or have such a widespread geographic distribution as *Austropuccinia* (=*Puccinia*) *psidii*, commonly known as myrtle rust (Beenken 2017). Originally infecting just three host species in South America (Winter 1884; Dale 1955), the number of *A*. *psidii* hosts has increased dramatically with its rapid global spread. While most rust species have few hosts (Hennen et al. 2005; Grgurinovic et al. 2006), *A*. *psidii* is known to infect more than 521 Myrtaceae species globally, across 78 genera (Giblin and Carnegie 2014; Roux et al. 2016; Soewarto et al. 2018; *Chapter 2*). The rust is now present in at least 27 countries on four continents (Simpson et al. 2006; Kawanishi et al. 2009; Carnegie et al. 2010; Giblin 2013; Roux et al. 2013; Carnegie and Pegg 2018), with a high likelihood of further spread into South East Asia (du Plessis et al. 2017). Effects on host plants include leaf distortion, defoliation, reduced reproductive output, dieback and death in highly susceptible individuals after repeated infections (Rayachhetry et al. 2001; Uchida et al. 2006; Carnegie et al. 2016).

Although recently described as a monotypic genus (Beenken 2017), cross-inoculation studies have shown some degree of host specificity between different biotypes (or strains) of *A*. *psidii* (Coutinho et al. 1998; de Carvalho et al. 1998; Graça et al. 2013; Silva et al. 2014). However, there is a wide host range reported in

Australia (Giblin and Carnegie 2014), Hawaii (Graça et al. 2011) and New Caledonia (Soewarto et al. 2018), despite the presence of only one biotype (the 'pandemic' biotype) of the rust (Machado et al. 2015). Some earlier studies have also shown low levels of specificity in tests with *Eucalyptus* species (e.g. De Castro et al. 1983). Recent molecular studies suggest that there are at least nine distinct genetic clusters ('biotypes') of *A*. *psidii* (Stewart et al. 2018). In Australia, several authors (Carnegie and Lidbetter 2012; Makinson 2018; Carnegie and Pegg 2018) have argued that maintaining a single strain of *A*. *psidii* is crucial to minimise its ecological impacts.

In Australia, *A*. *psidii* was first detected in 2010, on the central coast of New South Wales (NSW) (Carnegie et al. 2010). It rapidly spread north to Queensland in less than a year through a combination of anthropogenic spread via the nursery trade and natural spread via air-borne spores, and then to Victoria in 2011 via infected plants (Agriculture Victoria 2016). It was detected in the Northern Territory (Westaway 2016) and Tasmania (DEE 2016) in 2015. To date, it has not been found in South Australia or Western Australia. Australian ecosystems are dominated by Myrtaceae, and are therefore particularly vulnerable to *A*. *psidii* impacts ranging from species- to ecosystem-level (Makinson 2014). For example, *A*. *psidii* has dramatically reduced the distribution and abundance of the previously common rainforest understorey species *Rhodamnia rubescens* and *Rhodomyrtus psidioides* (Carnegie et al. 2016) as well as some *Gossia* species (Pegg et al. 2017). Approximately 19% of the native Myrtaceous species in Australia have either been tested for susceptibility to *A*. *psidii* infection or have been observed infected in the field (Carnegie and Lidbetter 2012; Morin et al. 2012; Pegg et al. 2012; Sandhu and Park 2013; Giblin and Carnegie 2014; Pegg et al. 2014a; Potts et al. 2016; Tobias et al. 2016), but almost 57% of species are expected to be exposed to *A*. *psidii* due to overlapping climate suitability

of host and pathogen, either now or in the future with climate change (Berthon et al. 2018). Currently 360 native taxa from 49 genera are known to be susceptible to the disease based on field observations or inoculation studies (Giblin and Carnegie 2014; Makinson 2018; *Chapter 2*), but only 182 species are confirmed to be infected in the field (Berthon et al. 2018).

There is considerable variation in the severity of infection between species to *A*. *psidii* (Morin et al. 2012; Sandhu and Park 2013). Some species show no signs of infection when exposed to the rust (completely resistant, e.g. *Eucalyptus cladocalyx*), while other species show consistently severe infection levels, with long-term exposure leading to mortality in the field (Carnegie et al. 2016; Pegg et al. 2017). Furthermore, considerable within-species variation has been shown to exist (Zauza et al. 2010; Pegg et al. 2014a; Lee et al. 2015; Potts et al. 2016; Pegg et al. 2018). Therefore, there is potential scope for identification of *A. psidii* resistance within species which will assist in the management of this invasive pathogen.

The aim of this study was to test the susceptibility to *A*. *psidii* of a range of previously untested Myrtaceous species/sub-species using a standard method developed by Berthon et al. (2018) (see details in Methods section). The species tested included ones that are (1) listed as endangered under national (EPBC Act 1999) or state-level legislation (NSW Threatened Species Conservation Act 1995 and QLD Nature Conservation Act 1992) and have a large overlap with *A*. *psidii* climatically-suitable areas in eastern Australia (Berthon et al. 2018); (2) not endangered but have a significant overlap of climatically-suitable area with *A*. *psidii*, (3) previously identified as a host species (*Chapter 2*) but need confirmation in order

to be included in the national host species list, and 4) included in a previously untested genus.

Methods

Selection of Species

Myrtaceous species were selected based on current or future overlap of climatically-suitable range with *A*. *psidii*, with particular emphasis placed on endangered species. Overlap was calculated based on Berthon et al.'s (2018) mapping of climatically-suitable areas for *A*. *psidii* and its potential hosts. However, Berthon et al. (2018) calculated host and pathogen overlap using extent of occurrence (EOO) as a measure of range [defined as the geographical extension encompassing all the areas where a certain species has been recorded (Gaston 1993)], while we used area of occupancy (defined as a subgroup of the EOO which excludes non suitable or not occupied areas (Gaston 1993)). This is a more precautionary measure, as it only considers areas with known populations as being at risk. This results in relatively (compared to EOO) small range sizes and relatively higher estimates of range overlap. It should be noted that three species (*Callistemon megalongensis* (now *Melaleuca megalongensis*), *Eucalyptus scopulorum* and *Leptospermum petraeum*) which have previously been shown to have significant range overlap with *A*. *psidii* (Kriticos et al. 2013), were not found to have host-pathogen range overlap by Berthon et al. (2018). However, these species were retained because if susceptible they may be particularly vulnerable to *A*. *psidii*, particularly *C*. *megalongensis* which is critically endangered (NSW Act 1995; EPBC Act 1999).

In total, sixteen endangered species were selected whose susceptibility to *A*. *psidii* is yet to be tested (Table 1). Furthermore, two of these species (*Triplarina nowraensis* and *T*. *imbricata*) are in a genus which has not previously been tested. In addition to the endangered species, three non-endangered species with large range overlaps with *A*. *psidii* were selected (Table 1; Appendix Table A5). Finally, two sub-species of two species (*Eucalyptus amplifolia* and *E*. *pachycalyx*) and two provenances of three species (*E*. *amplifolia*, *E*. *macarthurii* and *E*. *scoparia*) were selected (four sub-species and six provenances total) to test for intraspecific variation in susceptibility. It should be noted that *E*. *amplifolia* subsp. *amplifolia* is already present on the global host list, having been shown to be susceptible to *A*. *psidii* strains from Brazil (Zauza et al. 2010) and Japan (Kawanishi et al. 2009). However, its susceptibility to the *A*. *psidii* biotype present in Australia is yet to be tested. Information on the growth form, height, distribution and threat status of each species is provided in Table 1, and provenances (only for the non-endangered species) in Appendix (Table A6).

Table 1. Growth habit, reported height at maturity, distribution, threat status and group number of the 16 study species. NSW, New South Wales; QLD, Queensland; VIC, Victoria; NSW subdivisions: CC, Central Coast; CT, Central Tablelands; NC, Northern Coast; NT, Northern Tablelands; SC, Southern Coast; ST, Southern Tablelands. Threat status acronyms: EPBC, Environment Protection and Biodiversity Conservation Act; NSW, New South Wales Threatened Species Conservation Act; QLD, QLD Nature Conservation Act 1992; V, Vulnerable; E, Endangered; CR, Critically endangered. Least concern species have been evaluated but do not belong to any of the other categories, this is, they are at lower risk. Sources: PlantNet and Euclid.

Species	Growth habit	Height (m)	Distribution	Threat Status	Group
Callistemon megalongensis (Craven & S.M.Douglas) Udovicic & R.D.Spencer	Shrub	2.5	NSW (CT)	EPBC/NSW: CR	1
Eucalyptus amplifolia amplifolia Naudin	Tree	30	NSW	Least Concern	1
Eucalyptus amplifolia sessiliflora Naudin	Tree	30	NSW, QLD	Least Concern	1
Eucalyptus boliviana J.B.Williams & K.D.Hill	Mallee or tree	5 - 12	NSW (NT)	NSW BCA: V	2
Eucalyptus castrensis K.D.Hill	Mallee	8	NSW (NC)	NSW: E	1
Eucalyptus copulans L.A.S.Johnson & K.D.Hill	Tree	10	NSW (CT)	EPBC/NSW: E	1
Eucalyptus deuaensis Boland & Gilmour	Mallee or tree	5	NSW (ST)	Least concern	2
Eucalyptus fracta K.D.Hill	Tree or mallee	8	NSW (NC)	NSW BCA: V	2
Eucalyptus largeana Blakely	Tree	40	NSW (NC)	NSW: E	1
Eucalyptus macarthurii H.Deane & Maiden	Tree	40	NSW (CT), VIC	EPBC/NSW: E	1
Eucalyptus magnificata L.A.S.Johnson & K.D.Hill	Tree	15	NSW (NT), QLD	NSW: E	1
Eucalyptus ophitica L.A.S.Johnson & K.D.Hill	Tree	15	NSW (NC)	Least concern	2
Eucalyptus pachycalyx pachycalyx Maiden & Blakely	Tree	10	QLD	Least Concern	1
Eucalyptus pachycalyx waajensis Maiden & Blakely	Tree	10	NSW (NC), QLD	NSW/QLD: E	1
Eucalyptus parvula L.A.S.Johnson & K.D.Hill	Tree	15	NSW (ST)	EPBC: V/ NSW: E	1
Eucalyptus scoparia Maiden	Tree	15	NSW (NT), QLD	EPBC: V/NSW: E	1
Eucalyptus scopulorum K.D.Hill	Tree	8	NSW (NT)	Extrem. restricted	2
Kardomia prominens (A.R.Bean) Peter G.Wilson	Shrub	2.5	NSW (NC)	NSW BCA: E	2
Leptospermum petraeum Joy Thomps	Shrub	3	NSW (CT)	Least concern	2
Melaleuca irbyana R.T.Baker	Shrub/tree	8	NSW (NC), QLD	NSW BCA: E	2
Melaleuca tortifolia Byrnes	Shrub	4.5	NSW (NT)	Least concern	2
Micromyrtus blakelyi J.W.Green	Shrub	0.3 - 0.6	NSW (CC)	NSW BCA/EPBC: V	2
Triplarina imbricata (Sm.) A.R.Bean	Shrub	2.8	NSW (NC, CC)	NSW BCA/EPBC: E	2
Triplarina nowraensis A.R.Bean	Shrub	3.5	NSW (CC, SC)	NSW BCA/EPBC: E	2

Seed germination and growth of seedlings

Seed material for the endangered species was obtained from the Australian PlantBank at Mount Annan Botanical Gardens (NSW, Australia), while seeds for all other species were obtained from a commercial supplier (Australian Seed Company, Hazelbrook, NSW, Australia). Due to glasshouse space constraints, the 24 species were germinated, grown and inoculated in two separate groups (Table 1). The majority of species (N=20) were germinated on moist filter paper using 1% bleach solution to prevent mould growth. The exceptions were *T*. *nowraensis* and *T*. *imbricata* which were germinated on agar impregnated with gibberellic acid. Most species or sub-species (N=21) were germinated under a 12/12 light/dark cycle at 20°C, with the exception of *M*. *irbyana* which was germinated at 25°C under an 8/16 light/dark cycle due to different germination requirements.

For the first group of species, germinated seeds (one per pot) were planted into 100 mL pots (4 cm diameter, 8 cm depth), placed in trays and kept constantly moist. When the seedlings were 17 weeks old, they were re-planted into larger pots (13 cm diameter, 14 cm depth) and allowed to grow for an additional 17 weeks. Each pot contained 1.8 L of 80/20 sand/soil mix (ANL, North Ryde, NSW, Australia). For the second group of species, germinated seeds were planted straight into the 1.8 L pots and allowed to grow for 32 weeks. Seedlings were grown in a climate controlled glasshouse with the temperature set to 25^{o}C/18^{o}C daytime/night-time. To reduce any within glasshouse effects, plants were allocated new positions every four weeks. For the duration of the experiment pots were watered *ad libitum*, using an automatic mist watering system. When seedlings died of non *A*. *psidii* related causes (e.g.

transplanting) they were removed from the experiment. This resulted in low replicate numbers for some of the species.

Seedling inoculations and susceptibility scoring

Inoculations of seedlings with *A*. *psidii* were performed at the Plant Breeding Facilities at University of Sydney for the first group and the Plant Growth Facility at Macquarie University for the second group (Appendix Figure A7a). Inoculation occurred 34 weeks' post-germination for the former and 32 weeks post-germination for the latter. Inoculation was carried out using an aerosol hydrocarbon propellant pressure pack containing *A*. *psidii* urediniospores which were suspended in light mineral oil (20 mg urediniospores / 10 mL oil) and 0.05% of the surfactant Tween 20 (Appendix Figure A7b). A 'positive control' (*Syzygium jambos*, known to be highly susceptible) was also inoculated to ensure that the spores used were viable and that climatic conditions were optimal for infection (Appendix Figure A7c). Once inoculated, plants were placed within A1000 growth cabinets with CMP 6010 controller (Conviron, Winnipeg, MB, Canada) for 24 hours to ensure optimal humidity (95%) and temperature (21°C) conditions were maintained.

Several susceptibility scales have been developed to describe the range of plant responses to *A*. *psidii* infection. These different scales have recently been unified into one scale (Table 2, from Berthon et al. 2018) which was used for this study. A plant is considered resistant if it either shows no signs of infection, or exhibits only chlorotic or necrotic responses. In both cases, the development of the rust is inhibited, and no sporulation occurs, but a distinction is made based on the presence of tissue lesions from necrotic responses (Resistant vs Low Susceptibility,

Table 2). Cross-breeding (Junghans et al. 2003) and molecular studies (Tobias 2012) have shown that this response is genetically mediated, with individuals that show no signs of resistance (i.e. are highly susceptible) potentially lacking appropriate resistance genes (Kolmer et al. 2009). A plant is considered susceptible only if it shows fully developed uredinia. Some individuals show signs of necrosis as well as full development of spores, and this has been noted as moderate susceptibility by various authors (Morin et al. 2012; Sandhu and Park 2013; Pegg et al. 2014b). Highly susceptible individuals show no signs of necrosis or any other defensive response (Sandhu and Park 2013). In this study, infection was assessed on leaves and stems 14 days post-inoculation using the unified susceptibility scale (Table 2).

Table 2 A unified susceptibility scale created by Berthon et al. (2018) to describe the range of susceptibility reactions of hosts to *Austropuccinia psidii* (myrtle rust) based on previous studies. HR, highly resistant; R, resistant; MR, moderately resistant; RT, relatively tolerant; MS, moderately susceptible; S, susceptible; V, very susceptible; HS, highly susceptible; ES, extremely susceptible.

Score	Rating	Description	Equivalence for other scales			
			Morin1	Winzer 2	Pegg3	S & P [4]
0	Resistant	No infection	1	0		HR
1	Low	Infection but no sporulation	2	1		R
2	Medium	Infection and minimal sporulation	3-4	2-3	RT-MS	MR-MS
3	High	Infection and abundant sporulation on leaves, twigs and/or fruits	5	4-5	HS-ES	S-VS

[1]Morin et al. 2012, [2]Fernandez Winzer et al. 2018, [3]Pegg et al. 2014b, [4]Sandhu and Park 2013

The incidence of susceptibility for each species was counted as the number of individuals showing signs of infection (only individuals that showed moderate and high severity of infection). Differences in the proportion of susceptible individuals between species as well as between sub-species and provenances were tested using Kruskal-Wallis non-parametric analyses. Data analyses were carried out using Infostat statistical software (Di Rienzo et al. 2017).

Results

Of the 24 species/sub-species tested, 18 showed some degree of susceptibility to *A. psidii* (at least one individual infected per species) (Table 3; Appendix Figure A7e-f and A8a-g). Twelve of these susceptible species/sub-species are classified as endangered. In addition, one of the two species belonging to the genus *Triplarina* was found to be susceptible (*T. nowraensis*).

There were significant differences in the proportion of susceptible individuals between species ($H=128.25$; $p<0.001$). Six species were completely resistant showing no signs of infection (*E. fracta*, *E. ophitica*, *E. boliviana*, *E. scopulorum*, *M. blakelyi* and *T. imbricata*) while three species did not have any resistant individuals (*E. scoparia*, *M. irbyana* and *T. nowraensis*). Of the 15 susceptible species included in the analysis, 14 had some degree of within-species variation in susceptibility, with only *T. nowraensis* having the same level of susceptibility (medium susceptibility) across all individuals. Low replicate numbers for *K. prominens*, *M. blakelyi* and *T. imbricata* meant they were not included in the analysis to assess for differences in the proportion of susceptible individuals between species.

There were no significant differences in susceptibility between the *E. amplifolia* sub-species (H=1.17, p=0.495) and *Eucalyptus macarthurii* provenances (H=0.26, p=0.502). For two species, low replicate numbers in their sub-species (*E. pachycalyx waajensis*) and provenances (*E. scoparia* from the Northern Tablelands) meant sub-species and provenance differences were not tested.

Table 3 Number of individuals in each susceptibility category and the percentage of susceptible individuals (any individual that presented fully developed uredinia and was therefore rated as medium or highly susceptible) for all the species tested. The sixteen species/sub-species that are classified as endangered are represented in bold.

Species	Rep.	Number of individuals per susceptibility rating				Percentage susceptible
		Resistant	Low	Medium	High	
Callistemon megalongensis	14	1	3	9	1	71
Eucalyptus amplifolia amplifolia P1	16	7	8	1	0	6
Eucalyptus amplifolia amplifolia P2	15	8	5	2	0	13
Eucalyptus amplifolia sessiliflora	18	6	9	1	2	17
Eucalyptus boliviana	10	10	0	0	0	0
Eucalyptus castrensis	5	2	1	1	1	40
Eucalyptus copulans	17	1	0	2	14	94
Eucalyptus deuaensis	10	9	0	1	0	10
Eucalyptus fracta	10	10	0	0	0	0
Eucalyptus largeana	13	7	5	1	0	8
***Eucalyptus macarthurii* P1**	15	12	1	2	0	13
***Eucalyptus macarthurii* P2**	16	11	2	3	0	19
Eucalyptus magnificata	17	14	1	1	1	12
Eucalyptus ophitica	10	10	0	0	0	0
Eucalyptus pachycalyx pachycalyx	13	8	1	2	2	31
Eucalyptus pachycalyx waajensis	2	0	1	1	0	50
Eucalyptus parvula	15	3	0	7	5	80
***Eucalyptus scoparia* P1**	3	0	2	0	1	33
***Eucalyptus scoparia* P2**	7	0	2	4	1	71
Eucalyptus scopulorum	10	10	0	0	0	0
Kardomia prominens	1	0	0	1	0	100
Leptospermum petraeum	10	9	0	1	0	10
Melaleuca irbyana	10	0	0	8	2	100
Melaleuca tortifolia	10	1	9	0	0	0
Micromyrtus blakelyi	1	1	0	0	0	0
Triplarina imbricata	2	2	0	0	0	0
Triplarina nowraensis	11	0	0	11	0	100

Discussion

The aim of this study was to test the susceptibility of a range of previously untested Myrtaceous species/sub-species to *A*. *psidii*. We found that of the 24 species/sub-species tested, 18 were susceptible. Of these species, 12 are classified as endangered and one belongs to the previously untested *Triplarina* genus (*T*. *nowraensis*). These results mean that the number of *A*. *psidii* hosts in Australia has increased to 378 species and 50 genera.

Consistent with the findings of previous studies (Ferreira 1983; Dianese et al. 1984; Zauza et al. 2010; Morin et al. 2012; Sandhu and Park 2013; Potts et al. 2016), there was variation in susceptibility to *A*. *psidii* between species. Specifically, 18 species were susceptible and 6 were resistant. The most susceptible species (>90% susceptible) were *E*. *copulans*, *K*. *prominens*, *M*. *irbyana* and *T*. *nowraensis*. All of these species are considered endangered, which makes them the most warranting of conservation efforts. This is particularly true for *E*. *copulans*, for which there are only two known individuals in the field (PlantNET 2018). These conservation efforts are not only important at the species-level but also at the ecosystem-level, where declines in the distribution and abundance of species as a result of *A*. *psidii* could cause significant shifts in structure and function of ecological communities (Pegg et al. 2017).

Six of the 24 tested taxa were resistant to *A*. *psidii* infection. Of these, half are vulnerable (*E*. *fracta*, *E*. *boliviana* and *M*. *blakelyi*), one is endangered (*T*. *imbricata*) and the remaining two are not endangered (*E*. *ophitica* and *E*. *scopulorum*). However, two of these species had low replicate numbers (*M*. *blakelyi* and *T*. *imbricata*), so confirmation from field observations or testing a broader genetic base is needed. For

the remaining four species (*E*. *boliviana*, *E*. *fracta*, *E*. *ophitica* and *E*. *scopulorum*), we suggest that it is unlikely they will become infected in the field. This is because *Eucalyptus* species generally are most vulnerable to infection at the seedling life stage as they have a greater proportion of vulnerable new growth compared to mature plants (Ferreira 1983; Carnegie 2012). Due to the fact that the plants we tested were seedlings, it is extremely likely that the species we found to be resistant will be so across all life stages.

Contrasting the high proportion of resistant species found in this study (25%), Berthon et al. (2018) reported only 3% resistance (13/417) across all Australian species/subspecies either tested in the laboratory or observed in the field to date. The higher proportion of resistant species in our study could be related to the fact that they possess traits that could help protect them against infection. For example, we observed that the resistant species in our study tended to have higher leaf cuticle thickness than the susceptible species, although this was not specifically tested. This may have played a role in the resistance of the species as cuticle thickness has been associated with resistance of *Berberis* species to *Puccinia graminis* (wheat stem rust) (Melander and Craigie 1927). However, it is likely that many species present in the global susceptible species list also possess thick cuticles. Therefore, further studies are required to confirm if resistance is conferred externally by leaf and plant-level traits, internally by resistant genes or a combination of the two.

Consistent with previous studies (Morin et al. 2012; Potts et al. 2016), we found a higher level of resistance in *Eucalyptus* species compared to other genera. *Eucalyptus* species also had a large amount of within-species variation in susceptibility which may indicate an underlying diversity in resistance genes. A

genetic basis for resistance has been well studied in *E. globulus* (Butler et al. 2016; Tobias et al. 2016), *E*. *grandis* (Junghans et al. 2003; Mamani et al. 2010; Thumma et al. 2013), *E*. *pellita* (Santos et al. 2014) as well as in *Eucalyptus* hybrids (Alves et al. 2012), and breeding for resistance has been implemented as the most viable strategy to facilitate control of *A*. *psidii* in commercial plantations, mainly in Brazil (Furtado and Marino 2003; Glen et al. 2007, da Silva Guimarães et al. 2010) and Australia (Pegg et al. 2014a). For endangered species such as those tested in this study, the presence of resistance individuals is a promising avenue for restoration efforts within *A*. *psidii* affected areas. Further genetic testing to identify resistance genes in these species is vitally important as it is the first step in potentially introducing resistance genes into affected populations in the field.

Another conservation strategy that can be used to increase the resilience of affected populations in the field is to introduce different provenances of endangered species that are resistant to infection (Sniezko et al. 2014; Makinson 2018). Many studies have found differences between provenances or seedlots of the same species (Ferreira 1983; Dianese et al. 1984; Furtado and Marino 2003; Zauza et al. 2010; Pegg et al. 2014a; Lee et al. 2015; Potts et al. 2016; Pegg et al. 2018). While we have not covered the full breadth of possible sub-species or provenances for the species we tested, our preliminary results indicate consistency in the susceptibility between sub-species and provenances of *E*. *amplifolia* and *E*. *macarthurii*. However, low sample sizes combined with the high individual within-species variation seen in this study may be concealing underlying differences between sub-species and/or provenances. Considering that different provenances may be more exposed to *A*. *psidii* than others due to the climatic restrictions of *A*. *psidii*, investigating population

differences in susceptibility is an important area for future study (Berthon et al. 2018; Makinson 2018).

In this study, we have identified 18 previously untested species that are susceptible to *A*. *psidii* infection. However, it is important to note that although these species were found to be susceptible in controlled laboratory conditions, this may not necessarily translate to infection in the field. Natural variation in climate is unlikely to consistently produce optimal conditions for *A*. *psidii*, and optimal conditions may not always coincide with the presence of vulnerable new growth (Berthon et al. 2018). Therefore, plants under field conditions may have a greater chance of escaping infection even when found to be extremely susceptible in laboratory conditions. Field-based studies on the impacts of *A*. *psidii* on native vegetation have been limited to a few key species and ecological communities (Pegg et al. 2014b; Carnegie et al. 2016; Pegg et al. 2017). Therefore, the logical next step for research in this field is to link laboratory susceptibility findings to field observations which will enable us to determine the true impact of *A*. *psidii* on endangered species and ecological communities. Understanding the susceptibility of species in a field context is important for prioritising and administering management strategies to control the impact of *A*. *psidii* (Morin et al. 2012; Pegg et al. 2012; Pegg et al. 2014a,b). This is particularly important for endangered species, where implementing the best practices in terms of genetic seed sourcing for restoration and seed storage activities could be vital in preventing extinction relating to *A*. *psidii* (Taylor et al. 2016).

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CHAPTER FOUR

Plant architecture, growth and biomass allocation effects of the invasive pathogen myrtle rust (*Austropuccinia psidii*) on Australian Myrtaceae species after fire

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My contribution to the research and paper: Concept – 100%; Data collection – 80%; Analysis – 100%; Writing – 90%

Abstract

In 2010, the invasive fungus *Austropuccinia psidii*, which infects immature growth of Myrtaceous species and causes the disease known as myrtle rust, was detected in Australia. Many of Australia's Myrtaceous species occur within fire-prone vegetation and have the capacity to resprout after fire. Therefore, it is likely that new growth post-fire may be vulnerable to infection by *A*. *psidii*, with subsequent flow-on effects to species' persistence and community dynamics in fire-prone vegetation. The aim of this study was to test the impacts of *A*. *psidii* on native Australian Myrtaceae species after fire. We grew eight native species that are susceptible to myrtle rust in a glasshouse experiment before burning them and inoculating the post-fire new growth of half of the plants once with *A*. *psidii*. We assessed the effect of *A*. *psidii* on the architecture, growth and biomass allocation of our study species. Across all species, *A*. *psidii* significantly reduced the height but not the branching of the resprouting plants. As expected, specific leaf area was lower in inoculated plants although leaf biomass –contrary to expectation– was greater. Finally, biomass allocation did not significantly differ between infected and un-infected plants. From our results we can conclude that the combination of fire and *A*. *psidii* infection has significant impacts on plants at the species-level, which may have cascading effects at the community-level, especially after repeated infections which are known to cause greater impacts than a single infection event. Furthermore, these impacts may be exacerbated in the future under climate change, as the predicted increase in frequency and intensity of fires across much of Australia will result in more frequent new growth availability, making plants more vulnerable to *A. psidii* infection.

Key-words: disturbance, epicormic growth, leaf traits, native ecosystems, resprouters

Introduction

Exotic plant pathogens represent a global biodiversity threat because they act as strong evolutionary forces on individual species which in turn can shape the dynamics, structure and function of plant communities at a broader scale (Ellison et al. 2005, Loo 2009, Chakraborty 2013). Of these, fungal pathogens are regarded as the most important in terms of potential impacts (Van Alfen 2001). Infamous examples include *Puccinia graminis* (wheat rust), an invasive epiphytotic fungus that has decimated crops and put food security at risk since ancient Roman times (Schumann 1991); *Ophiostoma ulmi* (Dutch Elm disease) which has caused mortality in millions of North American and European elm trees (Strobel and Lanier 1981); and the soil-borne pathogen *Phytophthora cinnamomi*, which has caused extensive dieback and mortality of multiple hosts in *Eucalyptus* forests in Western Australia (Cahill et al. 2008). From these examples it is clear that we need to understand the potential impacts of newly introduced fungal pathogens in order to avoid or at least minimise the devastating effects that they may have on naïve native plant communities.

Austropuccinia (=*Puccinia*) *psidii*, also known as myrtle rust, eucalyptus rust and guava rust among other common names, is an invasive fungus that infects species exclusively in the Myrtaceae family. Originally from South and Central America (Winter 1884; Glen et al. 2007), it has now invaded several continents

including Australia in 2010 (Carnegie et al. 2010; Carnegie and Pegg 2018). In Australia, it was first detected in a cut-flower nursery on the Central Coast (60 km north of Sydney) of New South Wales (NSW), and an ultimately unsuccessful eradication attempt was mounted (Carnegie and Cooper 2011; Carnegie and Pegg 2018). Once *A. psidii* established in native ecosystems it rapidly spread along the east coast to southern NSW and northern Queensland (Pegg et al. 2014; Carnegie et al. 2016), and has since been detected in the Northern Territory (Westaway 2016), as well as Victoria (2011) and Tasmania (2015) (Berthon et al. 2018). It is yet to be detected in South or Western Australia, the latter of which is a biodiversity hotspot (Brooks et al. 2002). Currently, there are more than 539 susceptible species reported worldwide (Giblin and Carnegie 2014; Roux et al. 2016; Soewarto et al. 2018; *Chapter 2* and *3*), with ~378 of these being native to Australia (Giblin and Carnegie 2014; Makinson 2018; *Chapter 2* and *3*). For this reason, *A. psidii* poses a significant threat to Australian vegetation communities that have a significant Myrtaceous component (Wiltshire 2004).

The effects of plant pathogens at the species- and community-level is often exacerbated when interacting with a disturbance (Ayres and Lombardero 2000; Dale et al. 2001). For example, bark beetle-related mortality of pine trees in the United States significantly increased after severe drought (Lenart 2004). In Australia, one of the most common disturbances is fire (Gill 1975) which plays an integral role in shaping the structure and function of many native plant communities (Bradstock et al. 2012). In response to fire, a plant can either resprout (resprouters) or die and then regenerate from the seed bank (obligate seeders) (Clarke et al. 2013). A large proportion of Myrtaceous species in Australia, particularly from the *Eucalyptus* genus, are classified as resprouters, using resources stored in lignotuber (Pryor

1957; Graham et al. 1998; Noble 2001), epicormic (Kormanik and Brown 1967) or belowground structures (Lacey and Whelan 1976). Irrespective of what structure is used, resprouting species produce new growth which is particularly vulnerable to *A. psidii* infection (assuming the species is susceptible to the pathogen). The infection of this new growth, causing necrosis, distortion, and leaf and shoot death (Coutinho et al. 1998; Glen et al. 2007) can then influence the architecture, growth and biomass allocation of the plant. For architecture, the infection of apical meristems stimulates lateral bud growth which results in shorter and more highly branched plants (Booth et al. 2000; Glen et al. 2007) whereas leaf distortion, defoliation and branch dieback are all common ways *A. psidii* can affect the growth (due to reduced photosynthesis) and biomass allocation of a plant (Rayachhetry et al. 2001; Uchida et al. 2006; Carnegie et al. 2016). In our study, we used specific leaf area (SLA = leaf area/leaf dry weight) as a measure of the leaf area available for photosynthesis (Dijkstra and Lambers 1989; Evans and Poorter 2001). This is because the infection by *A*. *psidii* causes leaf distortion, which potentially increases the shade within the leaf reducing light absorption that is no longer available for photosynthesis (Dijkstra and Lambers 1989). Reduction in light absorption is also a consequence of leaf necrosis, another common *A*. *psidii* symptom (Rayachhetry et al. 1997; Uchida et al. 2006). Fungal pathogens are already known to cause reductions in rates of photosynthesis through leaf distortion and defoliation e.g. on field beans leaves in England (Malthus and Madeira 1993; Kuckenberg et al. 2009). Furthermore, the resprouting response of infected plants may be dampened as they also need to divert resources away from producing new growth towards defence (Growth-differentiation balance hypothesis; Herms and Mattson 1992). Therefore, continual re-infection of new growth after fire

is likely to result in carbohydrates depletion and increased mortality, with subsequent flow-on effects to community dynamics.

Although the impact to fire-recovering plants was identified as a potential threat to native Myrtaceae if *A. psidii* established in Australia (Grgurinovic et al. 2006), to date, only one study has tested the impact of *A*. *psidii* on native Australian Myrtaceae species (coastal heath species, Lennox Head, New South Wales) after fire (Pegg et al. 2018). This study found that the new growth produced by resprouting species after fire was highly susceptible to *A*. *psidii* infection, with some species (e.g. *Austromyrtus dulcis*, *Melaleuca quinquenervia*, *M*. *nodosa*) experiencing branch dieback after several reinfections. However, this study did not have control plots (due to fungicide use near waterways being restricted), making it difficult to quantify the actual impacts of *A*. *psidii* after fire. This means that despite the efforts of Pegg et al. (2018), the effect of an interaction between *A*. *psidii* and fire on native Australian species remains largely unknown (Makinson and Conn 2014). Therefore, the aim of this study was to address this knowledge gap by testing the impacts of *A*. *psidii* on susceptible native Australian Myrtaceae species after fire. To test this, we grew eight resprouting species in a glasshouse experiment which we then separated into two groups. One of these groups we inoculated a single time with *A*. *psidii* while the other was left as a control. We hypothesized that resprouting plants inoculated with *A*. *psidii* would show differences in 1) plant architecture, with reductions in height and increases in stem number compared to control plants; 2) growth, with reduced leaf biomass (due mainly to defoliation), and 3) biomass allocation, with reductions in specific leaf area (SLA) (due to increased leaf distortion) and leaf mass fraction (LMF) (due to both defoliation and resource diversion to defence) compared to control plants.

Methods

Study species

Using the most updated *A. psidii* national host species list (Berthon et al. 2018) and the NSW Flora Fire Response Database (Fire Ecology Unit, Office of Environment & Heritage, NSW, Australia), a list of native Australian species susceptible to *A*. *psidii* that resprout after fire was generated. This resulted in a list of 71 species, from which 12 species with contrasting resprouting strategies (basal or lignotuber) were selected (Table 1) based on the seed available from a commercial supplier (Nindethana Australian Seeds, Albany, WA, Australia). Of these 12 species, eight species had enough individuals (n>5) resprout after being burnt to be included in the final data analyses.

Table 1 List of study species with their respective resprouting origin (B = Basal, L = Lignotuber, E = Epicormic) and number of replicates per treatment. The species that had enough resprouting individuals (≥5) after being burnt to be included in the final data analyses are in bold.

Species	Resprout origin	Treated replicates	Control replicates
Angophora costata	B, E	9	9
Callistemon citrinus[1]	B, E	10	11
Callistemon rigidus[2]	B	10	10
Eucalyptus dalrympleana	L, E	5	6
Eucalyptus globoidea	L, E	6	6
Eucalyptus moluccana	B, E	10	10
Eucalyptus sieberi	L, E	1	1
Eucalyptus viminalis	L, E	6	6
Leptospermum juniperinum	L	-	-
Melaleuca nodosa	B, E	2	2
Melaleuca sieberi	B, E	2	1
Melaleuca styphelioides	B, E	10	11

[1] = *Melaleuca citrinus*, [2] = *Melaleuca rigidus*

Experimental design

Seeds for each species were germinated in aluminum trays lined with paper towels, which were moistened daily using 1% bleach solution to prevent mould growth. Once germinated, seedlings were transplanted at the stage of cotyledon emergence into 6 L pots (20 cm deep, 19.5 cm diameter) filled with 80:20 sand soil mix (Australian Native Landscapes Pty Ltd, North Ryde, NSW, Australia). We planted 20 replicates of each species that were divided evenly between the two treatments: not inoculated (control) and inoculated (treated) with *A*. *psidii* after resprouting post-fire. Within each pot three seedlings were planted as insurance against mortality. After four weeks of growth the medium size seedling was retained and the other two were removed. The plants were then left to grow for an additional 16 weeks (20 weeks total). They were grown in a climate controlled glasshouse (see Appendix Figure A10a) where the temperature was set to 22°C during the day and 20°C at night. Plants were mist watered twice daily for two minutes throughout the experiment. A slow release fertiliser (13N:4.8P:9.1K; Nutricote, Yates Australia Pty Ltd, Padstow, NSW, Australia) was applied (10 ± 0.2 g) at the start of the experiment to ensure plants were not nutrient limited.

After the 20 week growth period, the plants were defoliated to reduce flammability of the stems and avoid excessive burning of the plants, and all lateral branches were secured with wire next to the main stem to ensure homogenous burning of the plants. Plants were then burnt with a manual propylene TS4000 blowtorch (BernzOmatic, Columbus, OH, USA) at a distance of 1 cm, for 10 seconds every 90 degrees around the plant (40 seconds burning time per plant) (see Appendix Figure A10b).

Six weeks after burning, half the individuals of each species which had successfully resprouted (see Appendix Figure A10c) were inoculated with *A. psidii* using a hydrocarbon propellant pressure pack following standard procedures (Sandhu and Park 2013). Briefly, urediniospores were diluted in light mineral oil (Univar Solvent L naphtha 100, Univar Australia Pty Ltd) at a concentration of 2 mg mL^{-1}. Plants were inoculated a single time only. The inoculated plants were then kept in an A1000 growth cabinet with CMP 6010 controllers (Conviron, Winnipeg, MB, Canada) for 24 hrs to ensure optimal conditions for infection to occur were maintained (95% relative humidity at 20°C in darkness). Thereafter, plants from the different treatments were kept in separate glasshouses, with the same climatic conditions as described previously, to ensure no cross contamination occurred. Plants were also manually watered (the soil, not the foliage) to avoid the spores being washed off the plants. Fourteen days after inoculation, categories of infection were assessed for all inoculated plants following the protocols outlined in Pegg et al. (2014). Categories were defined as follows: *relatively tolerant* (RT) - pustules present in less than 10% of developing leaves and stems, *moderate susceptibility* (MS) - pustules present on 10-50% of developing leaves and juvenile stems, *high susceptibility* (HS) - pustules present on 50-80% of developing leaves and juvenile stems, and *extreme susceptibility* (ES) - pustules present on most leaves and stems, and presence of stem dieback.

Twelve weeks after inoculation, the SLA, maximum height and stem number of the resprouting new growth were recorded for all plants. SLA (leaf area/leaf dry weight) was measured for five randomly selected fully expanded leaves from each individual. Leaf area of the five leaves selected per individual was measured using a LI-3100C area meter (LI-COR, Inc. Lincoln, Nebraska, USA). Plants were then

harvested, washed free of soil and separated into their live leaf, stem and root biomass components. These biomass components (including SLA leaves) were then oven-dried at 70°C for 72 h and weighed using an electronic analytical balance (Sartorius Cubis Series, Goettingen, Germany). Finally, leaf mass fraction (LMF), stem mass fraction (SMF) and root mass fraction (RMF) were calculated as the proportion of the total plant dry mass in leaves, stems and roots, respectively (Peìrez-Harguindeguy et al. 2013).

Data analysis

Chi-square tests were used to determine species-level differences in *A*. *psidii* levels of infection. Linear mixed-models (LMM) were then used to test for *A*. *psidii* impacts on plant architecture, growth and biomass allocation after fire. Treatment (treated, control) and species were treated as fixed factors in the models. Data analyses were performed using InfoStat statistical software (Di Rienzo et al. 2017) which uses the R software package 'rlme' to perform the LMM (R Development Core Team 2018).

Results

Susceptibility to *A*. *psidii* differed significantly between species (χ^2_{14}=40.78, P<0.001). The most susceptible species were *E*. *globoidea* and *E*. *viminalis*, having 66.7% and 50% of individuals in the HS category, respectively (Fig. 1; Appendix Fig. A10h and j respectively). The least susceptible species were *E*. *dalrympleana*, *E*. *moluccana* and *M*. *styphelioides* with individuals only in categories RT and MS (levels 1 & 2; Appendix Table A9 and Fig. A10g,i,k).

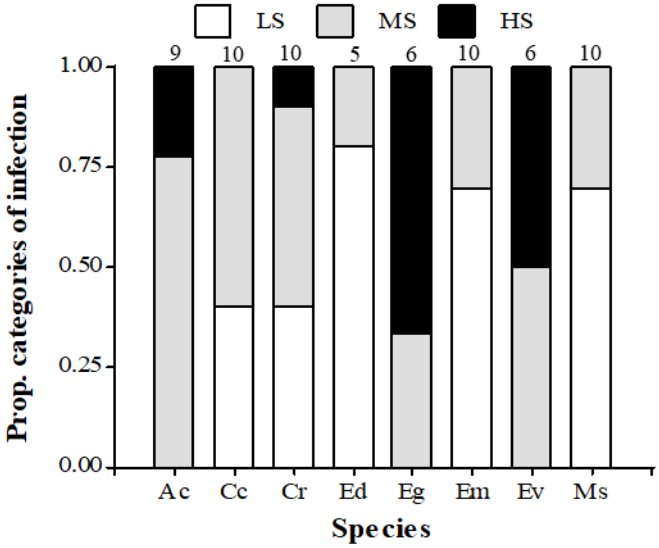

Fig 1 Categories of infection proportions for each species. Ac = *Angophora costata*, Cc = *Callistemon* (=*Melaleuca*) *citrinus*, Cr = *C.* (=*M.*) *rigidus*, Ed = *Eucalyptus dalrympleana*, Eg = *E. globoidea*, Em = *E. moluccana,* Ev = *E. viminalis*, Ms = *Melaleuca styphelioides.* Number of replicates tested can be found at the top of each species' bar. Categories of infection are defined as LS = low susceptibility, MS = moderate susceptibility, and HS = high susceptibility in the figure legend.

Impacts of *A. psidii* on plant architecture

There was no significant interaction between treatment and species for both the maximum height ($F_{7,119}$=1.14; P=0.341; Fig. 2a) and stem number ($F_{7,119}$=1.51; P=0.171; Fig. 2b) of the resprouting new growth. Across all species, the plants inoculated with *A. psidii* were significantly shorter than the control plants ($F_{1,119}$=12.97; P<0.001; Fig. 2a). In contrast, stem number of the resprouting plants did not differ between treatments ($F_{1,119}$=0.08; P=0.784; Fig. 2b). Finally, there were significant differences between species for both maximum height ($F_{7,119}$=12.33, P<0.001) and number of stems ($F_{7,119}$=3.90, P=0.001).

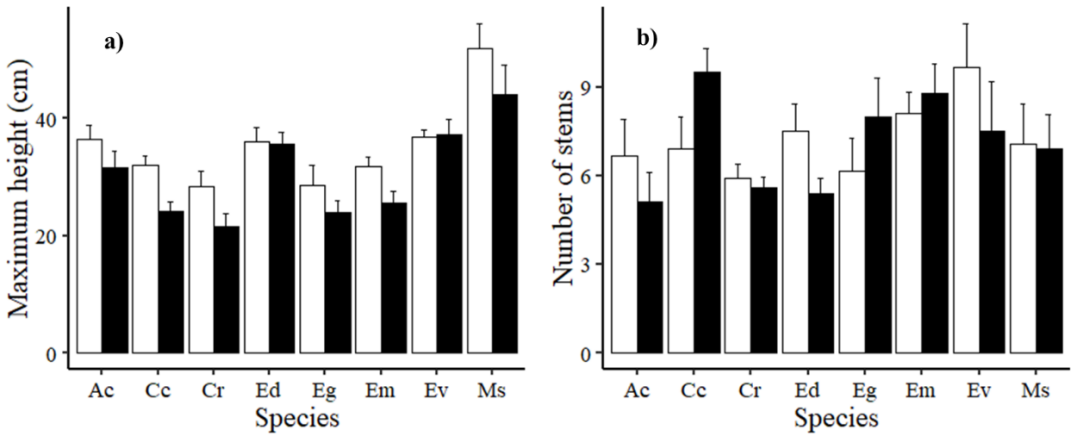

Fig 2a Maximum height and **b** stem number of the resprouting new growth of the control (white bars) and inoculated (black bars) plants of each species Ac = *Angophora costata*, Cc = *Callistemon* (=*Melaleuca*) *citrinus*, Cr = *C.* (=*M*.) rigidus, Ed = *Eucalyptus dalrympleana*, Eg = *E. globoidea*, Em = *E. moluccana,* Ev = *E. viminalis*, Ms = *Melaleuca styphelioides*. Vertical bars represent one standard error.

Impacts of *A. psidii* on growth

There was no significant interaction between treatment and species for leaf biomass ($F_{7,119}$=1.07; P=0.388; Fig. 3a), stem biomass ($F_{7,119}$=0.98; P=0.445; Fig. 3b), root biomass ($F_{7,119}$=0.96; P=0.460; Fig. 3c) or total biomass ($F_{7,119}$=1.14; P=0.343; Fig. 3d) of the resprouting plants. However, contrary to expectations leaf biomass was significantly greater in the inoculated plants ($F_{1,119}$ = 4.62; P = 0.034) compared to the control plants**.** There were no significant differences between treatments for stem biomass ($F_{1,119}$=0.83; P=0.364), root biomass ($F_{1,119}$=0.03; P=0.865) or total biomass ($F_{1,119}$=1.78; P=0.185). In contrast, there were significant differences between species for leaf biomass ($F_{7,119}$=20.81, P<0.001), stem biomass ($F_{7,118}$=15.77, P<0.001), root biomass ($F_{7,119}$=14.08, P<0.001) and total biomass ($F_{7,119}$=15.54, P<0.001).

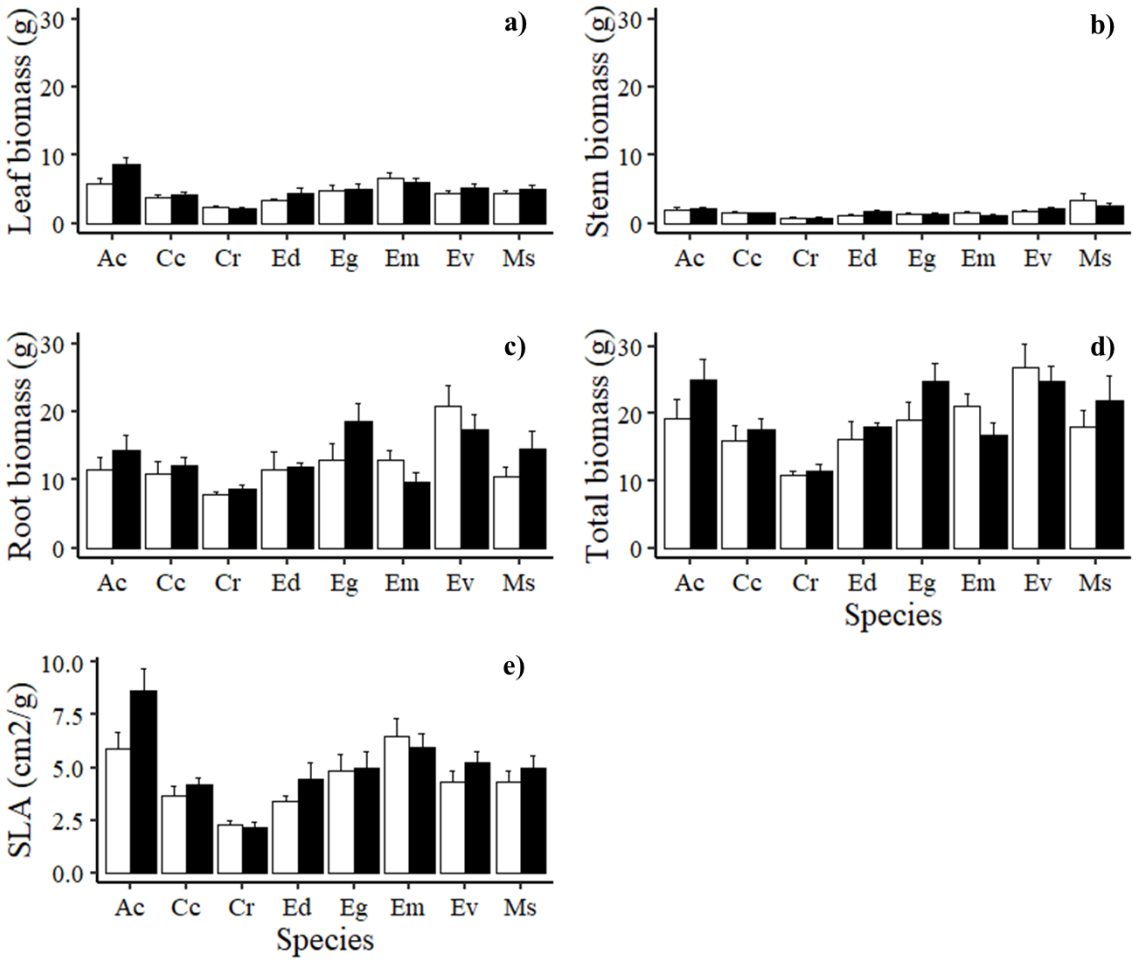

Fig 3a) Leaf biomass, **b)** stem biomass, **c)** root biomass, **d)** total biomass and **e)** specific leaf area (SLA), of the resprouting new growth of the control (white bars) and inoculated (black bars) plants for each species Ac = *Angophora costata*, Cc = *Callistemon citrinus*, Cr = *C. rigidus*, Ed = *Eucalyptus dalrympleana*, Eg = *E. globoidea*, Em = *E. moluccana,* Ev = *E. viminalis*, Ms = *Melaleuca styphelioides*. Vertical bars represent one standard error.

Impacts of *A. psidii* on biomass allocation

There was no significant interaction between treatment and species for SLA ($F_{7,115}$=1.65; P=0.128; Fig. 3e), LMF ($F_{7,119}$=1.79, P=0.095), SMF ($F_{7,118}$=1.12, P=0.359) or RMF ($F_{7,119}$=1.57, P=0.151) of the resprouting plants. As expected, the plants inoculated with *A. psidii* had significantly lower SLA ($F_{1,115}$=10.70; P=0.001)

than the control plants. In contrast, no significant treatment effects were found for LMF ($F_{1,119}$=1.61, P=0.207), SMF ($F_{1,118}$=1.28, P=0.261) or RMF ($F_{1,119}$=0.16, P=0.688. Finally, there were significant differences between species for SLA ($F_{7,115}$=26.14, P<0.001), LMF ($F_{7,119}$=13.17, P<0.001), SMF ($F_{7,118}$=9.45, P<0.001) and RMF ($F_{7,119}$=9.43, P<0.001).

Discussion

Disturbances can act in synergy to alter plant performance (Hobbs and Huenneke 1992). For example, fire can weaken plants physically and physiologically, leaving them vulnerable to herbivore and/or pathogen attack (Lombardero et al. 2006, Parker et al. 2006). For Australian vegetation, the potential interaction between fire and *A. psidii* and the impacts associated with it was foreseen by Grgurinovic et al. (2006) before *A. psidii* detection in Australia. Their argument was that the resprouting new growth of native Australian Myrtaceae species after fire would be particularly vulnerable to *A. psidii* infection (Grgurinovic et al. 2006). However, despite this logical link between the two disturbances, it is yet to be comprehensively tested and this study attempted to begin to address this knowledge gap. We found that *A. psidii* had a significant impact on all of our eight study species, with differences in plant height, leaf biomass and SLA between inoculated and control plants resprouting after fire. However, no individuals suffered mortality as a result of a single *A. psidii* infection event. This is consistent with previous studies that have found mortality in only highly susceptible species subject to repeated infections (Coutinho et al. 1998, Carnegie et al. 2016; Pegg et al. 2018). On a side note, susceptibility of four species (*C. rigidus*, *E. viminalis*, *M. sieberi* and *M. styphelioides*)

that were known to be susceptible to *A*. *psidii* in the most updated host species list (Berthon et al. 2018), but for which severity status had not been recorded, have been updated as a result of this study (Appendix Table A9). In addition, the severity status for *Eucalyptus globoidea* has also been updated to include the *moderately susceptible* score.

Previous studies have shown that one of the consequences of *A*. *psidii* infection is a shift in plant architecture towards shrub physiognomy which is characterized by reduced height and increased branching (Coutinho et al. 1998; Simeto et al. 2013). For our study species, we found that *A. psidii* reduced the maximum height of the plants but did not increase branching, either in terms of stem number or biomass. Fernandez Winzer et al. (2018) reported similar decreases in the height of infected plants for three coastal woodland species. However, these species showed considerable between- and within-species variation (*B. linifolia*: 15-30%, *L. laevigatum*: 15-30%, *M. quinquenervia*: 38-45%) in their height decreases, consistent with our study species (between: 0-25%, within: 7-14%). It has been suggested that the between-species variation may be a result of different susceptibilities, while the within species variation may be explained by differing climatic conditions, resource availability and number of reinfection events between studies (Smith 2006). Also consistent with our results, Fernandez Winzer et al. (2018) reported that branching was not altered by *A. psidii* after a single inoculation event in the three coastal woodland species mentioned above. Because of this it can be suggested that increased branching may only occur after successive reinfections of seedlings (that is, over a longer time period) or in plants at more advanced stages in their development (Fernandez Winzer et al. 2018).

Several studies on other plant pathogens have found that their impacts on growth (measured as height or wood volume) can be explained in part by the amount of total leaf area that is affected (Carnegie and Ades 2003; Rapley et al. 2009). This is because the leaf area of a plant determines its photosynthetic capacity and carbon assimilation (Evans and Poorter 2001). Partial or total plant defoliation is a common symptom of *A*. *psidii* infection that has been recorded in several species worldwide, including *Pimenta dioica* in Jamaica (Maclachlan 1938) and the United States (Marlatt and Kimbrough 1979), *M*. *quinquenervia* in the United States (Rayachhetry et al. 2001) and *Rhodamnia rubescens* in Australia (Carnegie et al. 2016). Considering this, we predicted that our inoculated plants would have reduced resprouting leaf biomass mainly as a result of defoliation. Surprisingly we found this not to be the case, with inoculated plants producing on average 15% more resprouting leaf biomass than the control plants across all species. This result is counterintuitive because although endophyte mutualistic fungi have been shown to trigger plant biomass increases, pathogenic fungi like *A*. *psidii* have not (Clay 1987, Waller et al. 2005). The increased resprouting suggests that the infected plants may run out of resources faster than the healthy plants in the longer term due to carbohydrate depletion, and this may be exacerbated by more frequent resprouting after repeated infections (McPherson and Williams 1998; Carnegie et al. 2016). However, it should be noted that the increases in resprouting leaf biomass were modest for most of our study species, with the exception of *A*. *costata* (47.5%). Given that we did not find a significant interaction in leaf biomass for treatment and species, we cannot draw conclusions related to species-specific impacts and leaf biomass in this study.

Consistent with our prediction of decreased SLA in *A*. *psidii* inoculated plants caused mainly by leaf distortion, we found that on average inoculated plants had 9%

lower SLA compared to the control plants. We think that this reduced SLA, in conjunction with increased defoliation, may have big impacts on photosynthesis capability as already mentioned, leading to carbohydrate depletion and mortality after repeated infections (McPherson and Williams 1998; Carnegie et al. 2016). Interestingly, we also found that the two species *E*. *dalrympleana* and *C*. *citrinus* that had the greatest reduction in SLA after *A*. *psidii* inoculation were not the most susceptible species, with no individuals having a high level of infection. This suggests that the susceptibility of a species to *A*. *psidii* may not translate directly to the impact that the pathogen has on the species. Furthermore, infection under artificial conditions may not be a good predictor of infection and subsequent impact in natural environments. This has been born out with studies showing various eucalypt species to be susceptible to *A*. *psidii* under artificial inoculation (Pegg et al. 2014; Lee et al. 2015) but with minimal evidence of disease in the same species in plantations or natural environments (Carnegie 2015; Carnegie and Pegg 2018).

In addition to replacing the lost foliage, infected plants need to also allocate resources to fight the disease (Herms and Mattson 1992; Hoffland et al. 1996). Considering this, we predicted that our inoculated plants would have reduced LMF as a result of both diversion of resources towards defence and defoliation. Contrary to expected, we did not find differences in LMF between inoculated and healthy plants. This was surprising as well given that the increase in resprouting leaf biomass was not reflected in an increase in LMF. This may be because the root biomass component of our plants was significantly larger than the leaf biomass component (because the aboveground biomass components of our plants were burnt) thus overwhelming any shift that may have occurred in leaf biomass allocation. Therefore, we suggest that future studies assessing the impact of *A. psidii* on biomass allocation should be longer

in duration than the current study so the resprouting aboveground biomass components are given more of a chance to reach a size where their allocation shifts are detectable relative to the root biomass. Nonetheless, it is interesting to highlight that the negative effects caused by *A*. *psidii* on resprouting plants architecture, growth and SLA occurred after only a single fire event and a single *A*. *psidii* inoculation, suggesting that greater impacts can be expected in natural conditions. For example, it is likely that the persistence of susceptible Myrtaceae species in the community will be reduced when having to cope with defoliation from both fire and *A*. *psidii* repeated events.

This study has shown that the combination of fire and *A*. *psidii* can impact the architecture, growth and SLA of native Australian Myrtaceae species. These results may have significant implications for the structure and function of Australian vegetation communities including shifts in plant physiognomy, community composition and competitive interactions. However, this study was only short term so we suggest longer-term studies, including field experiments, are needed to fully understand the impact of *A*. *psidii* after fire at both the species- and community-level. In terms of managing these impacts, options are scarce as fire is a common natural disturbance in many Australian plant communities and controlling *A*. *psidii* at a community-level is unrealistic. Unfortunately, this means that cascading effects on co-occurring non-Myrtaceous vegetation (*Chapter 4*) and fauna (e.g. mammals and birds nesting on susceptible species or foraging their fruits; Lepidoptera and Coleoptera with obligate plant-insect relationships; Makinson 2018) should be expected. Therefore, the focus should be shifted towards preventing *A. psidii* spreading to unaffected areas such as South and Western Australia. Biosecurity actions, such as restrictions on the import of susceptible Myrtaceae species, are

already in place for this purpose. To complement these biosecurity measures, monitoring programs to allow the early detection of *A*. *psidii* in the field are crucial but have currently not been implemented. Although not tested by this study, the interaction between fire and *A. psidii* may also have large implications for Australian vegetation communities in the future. For example, the threat posed by *A. psidii* may be exacerbated in the future with rising temperatures and shifting rainfall patterns associated with climate change resulting in more frequent fires in southern Australia (Williams et al. 2009). However, these higher temperatures coupled with shifting rainfall patterns will likely result in drier conditions (Cary et al. 2012), which in turn may be sub-optimal for *A. psidii* spore germination. From these different scenarios it is clear that the impacts of *A. psidii* on Australian vegetation after fire in the future will likely be dependent on complex interactions between climate change associated environmental variables.

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CHAPTER FIVE

Direct and indirect community effects of an invasive plant pathogen (*Austropuccinia psidii*) in eastern Australian rainforests

This chapter has been prepared for publication in *Journal of Applied Ecology*.

My contribution to the research and paper: Concept – 80%; Data collection – 90%; Analysis – 90%; Writing – 90%

Summary

1. In 2010, the invasive fungal pathogen *Austropuccinia psidii* was detected in Australia threatening Australian vegetation communities as it infects species from the dominant Myrtaceae family. In this study, we assessed the community-level impacts of *A. psidii* resulting from the infection of two highly susceptible rainforest species: *Rhodamnia rubescens* and *Rhodomyrtus psidioides*.

2. A large scale field experiment was performed in New South Wales, Australia, with three sites selected per study species. For *R. rubescens*, 20 plots, each containing an adult tree, were established per site with each plot designated one of four treatments: fungicide spray of the understorey only, canopy only, both or none (control). For *R. psidioides*, 30 plots containing seedlings were established per site, with each plot designated one of two treatments: understorey fungicide spray or non-spray (control). Richness and abundance of co-occurring understorey species was assessed every four months for a 24-month period. In addition, for *R. rubescens* plots changes in canopy transparency were assessed.

3. We found that the *R. rubescens* control canopy plots had greater canopy transparency (19%) which was associated with a reduction in the richness (8%) and total abundance (46%) of co-occurring understorey species. For treated canopy plots, richness was similar but total abundance increased (47%) in fungicide treated understorey plots. In contrast, in the *R. psidioides* plots overall understorey species richness and abundance did not differ between control and treated plots. However, plots treated with fungicide had significantly greater abundance of *R. rubescens* and *R. psidioides* seedlings (112% and 190%, respectively) compared to control plots.

Over the study period *R. rubescens* trees did not produce any viable seed irrespective of the fungicide treatment.

4. *Synthesis and applications:* We have shown that in a short time period (24 months) infection of two mid-canopy species by *A. psidii* has resulted in changes in species richness and abundance in Australian rainforest communities. These changes have arisen through both direct effects (changes in understorey species richness and abundance, and mortality of adult trees and seedlings), and indirect effects (through changes in light availability due to canopy dieback). We suggest that more dramatic changes in vegetation composition are likely over the longer-term. Further, local extinctions of these two highly-susceptible rainforest species are likely due to their inability to produce viable seeds. Conservation efforts should focus on seed and tissue collection as well as identification of less susceptible plants for future propagation and translocation action.

Key-words: biodiversity; environmental impacts; forest pathogens; invasive species; myrtle rust; *Puccinia psidii*

1. Introduction

Invasive plant pathogens are a major threat to biodiversity globally as they are able to severely impact the composition, structure and function of vegetation communities (Ellison et al. 2005; Loo 2009; Roy et al. 2014). Among the various groups of pathogens which infect plants, fungal pathogens are regarded as the most important in terms of potential impacts (Van Alfen 2001). Notable examples of invasive fungal pathogens include *Ophiostoma ulmi*, the cause of Dutch Elm disease, which is responsible for the mortality of millions of trees in North America and Europe (Strobel and Lanier 1981); *Phytophthora cinnamomi*, a soil-borne pathogen which has caused extensive dieback of Proteaceae woodlands (especially *Banksia* spp.) in Western Australia (WA) as well as impacts to other vegetation communities (Burguess et al. 2009); and *Cronartium ribicola* or white pine blister rust, whose main host *Pinus albicaulis* has been declared endangered in Canada as a result of the pathogen's severe impacts (Government of Canada 2012; Keane et al. 2012).

In 2010, the invasive fungal pathogen *Austropuccinia psidii* (myrtle rust), native to South and Central America (Winter 1884; Glen et al. 2007), was first detected on *Agonis flexuosa* in a plant nursery on the Central Coast of New South Wales (NSW), Australia (Carnegie et al. 2010). An eradication attempt was unsuccessful (Carnegie and Cooper 2011), and myrtle rust has since spread rapidly through eastern Australia, initially via anthropogenic activity (e.g. movement of infected plants) then more commonly via air-borne spores. *Austropuccinia psidii* poses a significant threat to Australian vegetation communities as it infects the young growing tissue of species in the Myrtaceae family, one of the dominant plant families in Australia (Glen et al. 2007). Infection often results in leaf distortion (Coutinho et al.

1998), branch dieback (Pegg et al. 2018), altered plant architecture (Takahashi 2002; Fernandez Winzer et al. 2018, *Chapter 4*), reduced flower and fruit production (Smith 1935, Carnegie et al. 2016; Pegg et al. 2018) and mortality in highly susceptible species (Carnegie et al. 2016). The invasive potential of *A*. *psidii* is evident by its successful invasion of a number of countries across several continents (see Carnegie and Pegg 2018, Table 1 for a complete list) including Jamaica (MacLachlan 1938), U.S.A (Marlatt and Kimbrough 1979), Japan (Kawanishi et al. 2009), China (Zhuang and Wei 2011), South Africa (Roux et al. 2013), New Caledonia (Soewarto et al. 2018), Indonesia (McTaggart et al. 2016), Singapore (du Plessis et al. 2017) and more recently New Zealand (DOC 2017). While most fungal pathogens infect just a handful of species, *A*. *psidii* is known to infect 539 species worldwide from 79 different genera (Giblin and Carnegie 2014; Makinson 2018; Soewarto et al. 2018; *Chapter 2 and 3*). In Australia alone, there are 378 native species from 50 genera known to be susceptible through susceptibility tests and/or field observations (Giblin and Carnegie 2014; Makinson 2018a; *Chapter 2 and 3*). As Australia is home to 40% of the global Myrtaceae species (2,250) and 60% of the genera (80) (Grattapaglia et al. 2012; Makinson 2014), it is vital that the ecological impacts of *A*. *psidii* are well understood.

Despite *A. psidii* being established in Australia for almost eight years, there have been few studies on its impact on native vegetation, as research has focused more on commercial plant industries than on environmental impacts (Carnegie and Pegg 2018). For example, the lemon myrtle (*Backhousia citriodora*) industry has been severely affected by *A. psidii* due to reduced leaf production and the need for fungicide application halting attempts for organic production (Doran et al. 2012; Lancaster et al. 2016). Globally, the impacts of *A*. *psidii* on native vegetation

communities are poorly understood, with only a few studies conducted in Hawaii (Loope and La Rosa 2008) and Australia that focused on individual species only. In Australia, study efforts have been concentrated in Queensland (QLD) and NSW where *A*. *psidii* has been established for eight years. In southern QLD, the already endangered species *Gossia gonoclada* (~300 individuals) was shown to be susceptible to infection prior to *A*. *psidii* arrival and is considered to have a limited chance of survival in the wild (Taylor 2013). Pegg et al. (2017) reported that another four native species (*Archirhodomyrtus beckleri*, *Decaspermum humile*, *Gossia hillii* and *Rhodamnia maideniana*) are following a similar trajectory in QLD. In NSW, Carnegie et al. (2016) studied the impacts of *A*. *psidii* on two highly susceptible rainforest species, *Rhodamnia rubescens* and *Rhodomyrtus psidioides*. They found that infected adult trees of these species had increased canopy transparency and higher mortality after repeat infections. Furthermore, despite these species being widespread in NSW prior to *A*. *psidii* arrival, they have now been preliminary listed as critically endangered as a result of *A*. *psidii* (NSW Scientific Committee 2017). Although these handful of studies have shown the detrimental impacts of *A*. *psidii* at the species-level, the potential flow-on community-level impacts are yet to be assessed. Therefore, the aim of this study was to assess the community-level impacts of *A*. *psidii* as a result of infection of two highly susceptible rainforest mid-canopy species. The community-level impacts we assessed were changes in species richness and abundance of the co-occurring understorey species and the recruitment of *R*. *rubescens* and *R*. *psidioides* seedlings. We hypothesized that *A*. *psidii* infection will result in increased defoliation and mortality (both resulting in increased canopy transparency) of *R*. *rubescens* trees. We predicted that as a consequence of the increased light availability, there would be an increase in the richness and abundance

of co-occurring understorey vegetation. We also predicted that *A*. *psidii* infection would directly reduce the recruitment of *R*. *rubescens* and *R*. *psidioides* seedlings in the understorey, resulting in increases in the richness and abundance of co-occurring understorey species.

2. Material and methods

2.1 Study species

Two rainforest species (*R*. *rubescens* and *R*. *psidioides*) known to be highly susceptible to *A*. *psidii* were selected as the focal species for this study. Both are mid-storey species with coastal distribution, that were once widespread in New South Wales to South East Queensland, but currently their distribution is being reduced by *A*. *psidii* resulting in them being preliminary listed as critically endangered (NSW Scientific Committee 2017).

Rhodamnia rubescens (Bentham) Miquel, known as 'brush or scrub turpentine', is a shrub/small tree that can reach 30 m in height (Floyd 1989; Robinson 1991). It is a pioneer species present in most rainforest community types from Batemans Bay (NSW) to Maryborough (QLD) (Floyd 1989). *Rhodomyrtus psidioides* (G.Don) Benth., commonly known as 'native guava', is a shrub/small tree that can reach 12 m in height (Floyd 1989). It is present in littoral and subtropical rainforest as well as wet sclerophyll forest, from Gosford (NSW) to Maryborough (QLD) (Floyd 1989).

2.2 Site selection and locations

In order to study the impacts of *A. psidii* on eastern Australian rainforest communities, field sites containing significant natural stands of the two study species were selected. Sites were selected based on previous field assessments by Carnegie et al. (2016). Three sites were selected for each of our study species, all within NSW. For *R. rubescens*, sites were located in Royal National Park (34º08.324'S, 151º01.676'E; subtropical and warm temperate rainforest), Wambina Nature Reserve (33º29.155'S, 151º26.186'E; warm temperate rainforest) and Olney State Forest (33º13.497'S, 151º25.795'E; wet sclerophyll forest) (Fig. 1). For *R. psidioides*, sites were located in Bundjalung National Park (28º51.639'S, 153º35.713'E; littoral rainforest), Wamberal Lagoon Nature Reserve (33º25.128'S, 151º27.471'E; littoral rainforest) and Shelly Beach in the Ballina Shire (28º51.656'S, 153º35.706'E; littoral rainforest) (see Fig. 1).

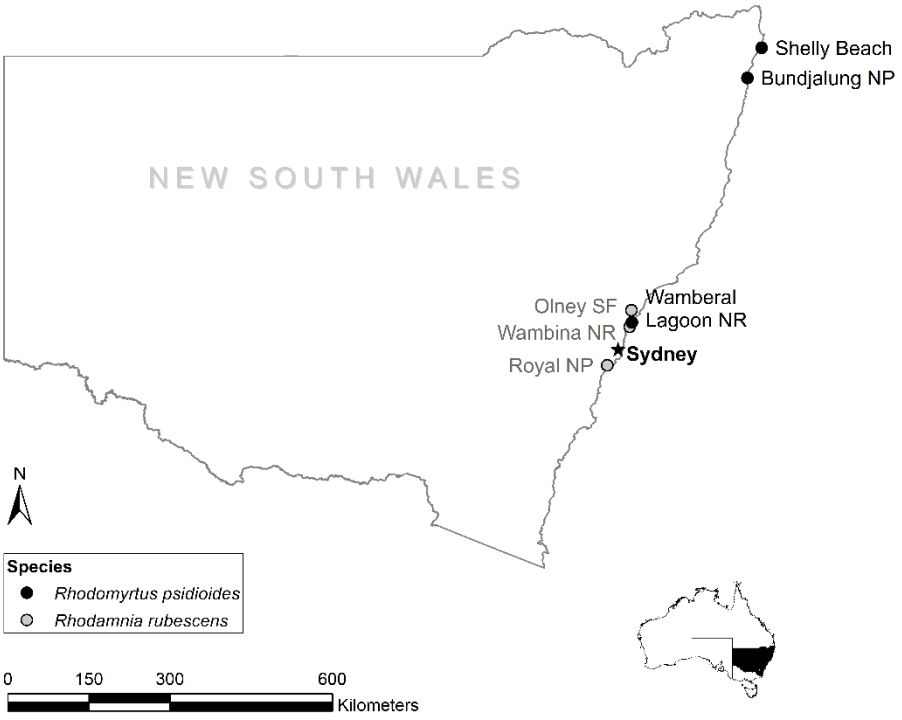

Fig 1 Map of New South Wales (Australia, bottom right) showing the three field sites of each of the two study species *R*. *rubescens* (grey circles) and *R*. *psidioides* (black circles).

2.3 Experimental design

The field experiment was conducted over a 24-month period between May 2016 and May 2018. For *R*. *rubescens*, 20 trees of approximately 1-5 m in height (taller trees would be too difficult or impossible to spray) were selected at each of the three sites. All the selected trees showed previous signs of *A*. *psidii* infection. A 2 m x 2 m plot was then established around each tree. One of four treatments was randomly assigned to each plot (five plots per treatment per site). The treatments were: 1) control (not sprayed) tree, control understorey (Ct:Cu) (Fig. 2a); 2) control tree, treated (sprayed) understorey (Ct:Tu) (Fig. 2b); 3) treated tree, control understorey (Tt:Cu) (Fig. 2c); and 4) treated tree, treated understorey (Tt:Tu) (Fig. 2d). In the

Tt:Cu plots, sheets of inert black plastic were used to avoid fungicide dripping from the sprayed trees onto the understorey vegetation (Fig. A12d). It should be noted that the diameter of the *R. rubescens* trees at breast height was measured to ensure no significant differences in tree size existed between treatments within sites (Olney SF: $F_{3,16}$=0.27, p=0.849; Royal NP: $F_{3,16}$=0.70, p=0.566; Wambina NR: $F_{3,16}$=3.02, p=0.061).

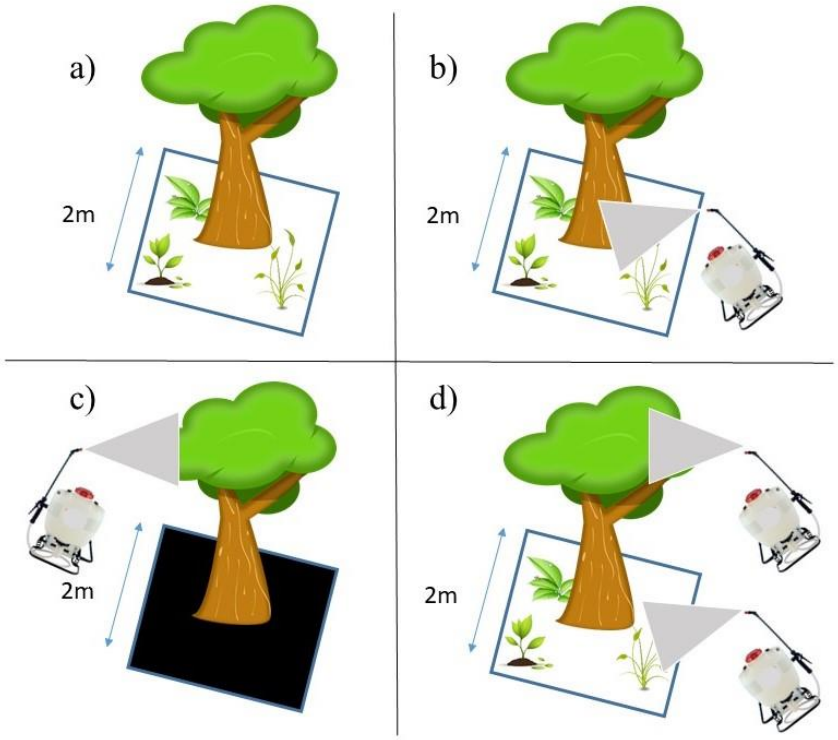

Fig 2 Spray treatments applied to *Rhodamnia rubescens* plots: **a)** control (not fungicide sprayed) tree, control understorey (Ct:Cu); **b)** control tree, treated (fungicide sprayed) understorey (Ct:Tu); **c)** treated tree, control understorey (Tt:Cu) with sheets of inert black plastic covering the understorey to avoid fungicide dripping from the sprayed trees; and **d)** treated tree, treated understorey (Tt:Tu).

For *R*. *psidioides*, a different experimental design was employed as we were unable to find any adult trees surviving. Therefore, plots were selected based on the presence of seedlings. Ten 2 m x 2 m plots were established at each site, and two treatments were applied: 1) control (understorey not sprayed); 2) treated (understorey sprayed). Five plots were assigned to each treatment per site.

Across all sites, irrespective of the study species, treated plots were sprayed to the point of run-off with the fungicide triadimenol 50 mL/100 L (approved for use in Australia in *A*. *psidii* control http://permits.apvma.gov.au/PER82008.PDF) using a 15 L manual pressurized backpack spray unit (Model 425LC, Solo, Dandenong South, VIC, Australia; Fig. A12e). Fungicide application was performed every 3-4 weeks depending on climatic conditions, for the period of the study.

Tree and understorey species assessments

The following variables were measured every four months (six census times over the two years) at the plot-level: 1) canopy transparency of the *R. rubescens* trees; 2) richness and total abundance of understorey species; and 3) abundance of our study species seedlings. It should be noted that these variables were not measured in November 2016 for the *R. rubescens* plots because of time constraints. Canopy transparency, which is defined as the amount of sunlight that penetrates the living canopy, was used as a proxy for defoliation (Schomaker et al. 2007). As no other cause of defoliation (e.g. drought or herbivores) was observed during the study we attributed any canopy transparency changes to *A. psidii* infection, as previously done by Carnegie et al. (2016) and Pegg et al. (2017). Canopy transparency was assessed using canopy pictures taken with a fish eye lens camera, positioning the tripod in the

same location in each plot every census time. Images were analyzed with the Gap Analyzer software (Version 2.0, Simon Fraser University, Burnaby, British Columbia, Canada). In each plot, every seedling was identified to the species-level by reference to relevant field guides (e.g. Robinson 1991), and its abundance visually assessed.

In addition to the measured variables described above, the presence of active rust (yellow urediniospores) on new flush, flowers and fruits (if present) was recorded for all trees and seedlings of the study species.

2.4 Data Analysis

To determine the effect of *A. psidii* on the 1) canopy transparency of *R. rubescens* trees, 2) richness and total abundance of co-occurring understorey species of *R. rubescens* and *R. psidioides* plots (not including our study species) and 3) abundance of *R. rubescens* and *R. psidioides* seedlings, linear mixed models (LMM) with repeated measures were used. The fixed factors in these models were spray treatment (four levels for *R. rubescens* plots, two levels for *R. psidioides*) and time (four levels for *R. rubescens*, five levels for *R. psidioides*). The random factors in the model were site (three levels per study species) and plot (60 levels for *R. rubescens*, 30 for *R. psidioides*) which was nested within site. To select the model with the most appropriate covariance structure (e.g. autoregressive, compound symmetry), the Akaike Information Criteria (AIC) was used. Data used in the analyses were calculated as the proportional change in the variable of interest in relation to the reference value at the beginning of the experiment before the application of fungicide (census time 1). Least significant difference post-hoc analyses were carried out if there was a significant interaction between treatment and time ($\alpha = 0.05$).

For the canopy transparency analysis of the *R. rubescens* trees, the plots with control (Ct:Cu, Ct:Tu) and treated (Tt:Cu, Tt:Tu) canopies were compared. To test the indirect effect of myrtle rust through changes in light availability, understorey species richness and total abundance were compared between canopy treated plots (Tt) and canopy control plots (Ct), both with treated understorey (Tt:Tu and Ct:Tu). To test the direct effect of myrtle rust on understorey vegetation, we compared understorey control and treated plots below canopy treated trees (Tt:Cu and Tt:Ct).

Finally, to determine if changes in the canopy transparency of *R. rubescens* trees were driving shifts in the richness and abundance of co-occurring understorey species, ordinary least-squares regression models were generated.

Data analyses were performed using Infostat software (Di Rienzo 2017), which uses the R software package 'rlme' to perform the LMMs (R Development Core Team 2017). When necessary, to satisfy the normality requirements and fulfil the assumptions of LMM, a square root transformation was used.

3. Results

Rhodamnia rubescens plots

As predicted, the canopy transparency of the control trees was significantly greater than that of the fungicide-treated trees for census times 2 to 5 (interaction: $F_{3,174}$=9.583, p<0.001; Fig. 3a and Fig. A12a for a close-up picture of an infected leaf). This interaction is explained by the increasing difference in canopy transparency between treatments through time.

We identified 155 understorey species in the *R. rubescens* plots, none of which were Myrtaceae (with the exception of *R. rubescens*; Table A11). In terms of indirect effects of *A*. *psidii*, contrary to our prediction the treated canopy plots (Tt:Cu, Tt:Tu) had greater species richness ($F_{3,84}$=4.73, p=0.004; Fig. 3b) than the control canopy plots (Ct:Cu, Ct:Tu), for census times 4 and 5 (on average). Similarly, the treated canopy plots had greater total abundance ($F_{3,83}$=4.15, p=0.009; Fig. 3c) than the control canopy plots for census times 2 to 5. For the control (untreated) canopy plots we found that the greater species richness ($F_{1,57}$=4.57, p=0.037; slope=-0.236; R^2=0.03; Fig. 4a) and total abundance ($F_{1,57}$=6.43, p=0.014; slope=-0.764, R^2=0.08; Fig. 4b) was associated with the increased canopy transparency of the *R. rubescens* trees.

In terms of direct effects of *A*. *psidii* on the understorey species richness, we found a significant interaction between treatment and time for the treated canopy plots (Tt:Cu, Tt:Tu; $F_{3,84}$=2.91; p=0.039; Fig. 3d). However, there were no significant differences between treatments at each time. The interaction is therefore explained by the control understories (Tt:Cu) having greater (although non-significant) richness initially, but the fungicide sprayed understories (Tt:Tu) having greater richness (non-significant) at the end of the experiment. Treated understories (Tt:Tu) had greater total abundance (interaction: $F_{3,84}$=3.02; p=0.034; Fig. 3e) than control understories (Tt:Cu) for census times 2 to 5. This interaction is explained by the increasing difference in total abundance between treatments through time. It must be remembered that these results are obtained without considering *R*. *rubescens* abundance to avoid the possible strong effects of the study species.

To assess the direct effect of *A*. *psidii* on *R*. *rubescens* seedling abundance, we compared treated and control understorey for treated canopies (Tt:Tu, Tt:Cu). The abundance of *R*. *rubescens* seedlings was significantly reduced in the control compared to treated understorey at census times 3, 4 and 5 ($F_{3,86}$=3.29; p=0.025; Fig. 3f).

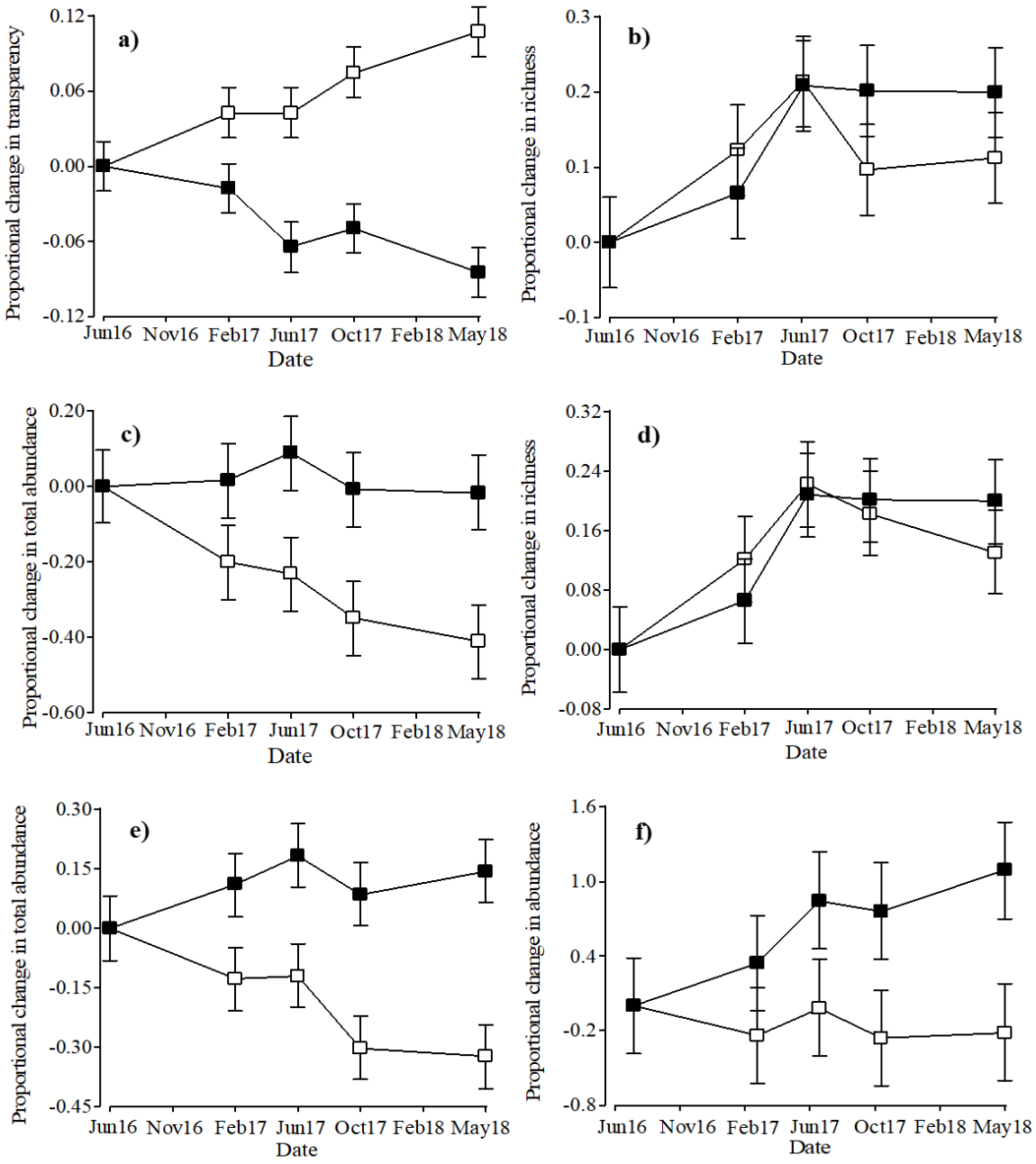

Fig 3 Proportional change through time in the *Rhodamnia rubescens* plots for (**a**) canopy transparency of *Rhodamnia rubescens* trees, (**b, d)** co-occurring understorey species richness, (**c, e)** co-occurring understorey total abundance and (**f)** abundance of *Rhodamnia rubescens* seedlings abundance. Panels **b** and **c** have different fungicide treatments for canopy while **d** and **e** have different fungicide treatments for understorey. For panels **a**, **b** and **c**, the white squares represent the control canopy plots while the black represent the treated canopy plots. For panels **d**, **e** and **f**, the white squares represent the control understorey plots while the black squares represent the treated understorey plots.

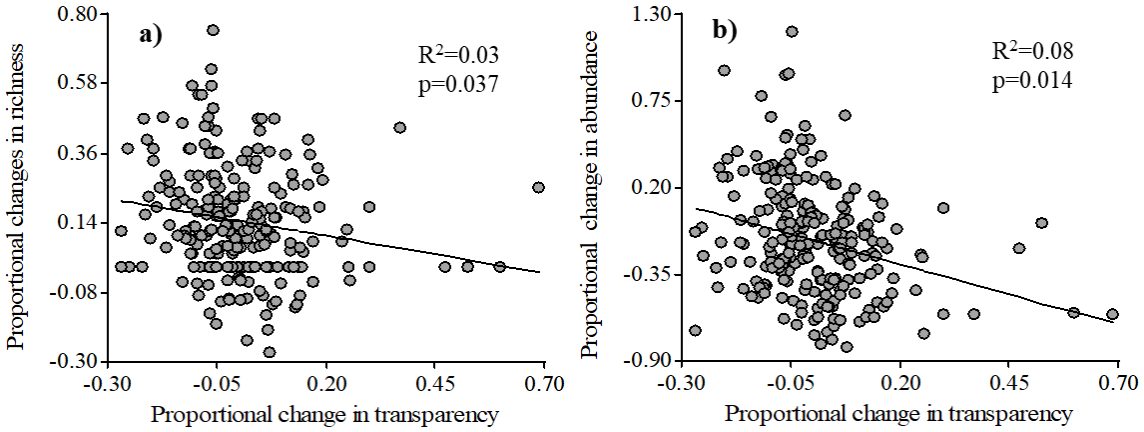

Fig 4 The relationship between proportional change in canopy transparency through time of *Rhodamnia rubescens* trees and (**a**) proportional change in understorey richness, and (**b**) proportional change in understorey total abundance.

Of the 60 *R. rubescens* trees assessed, two experienced mortality (both controls) while nine produced flowers (five controls Fig. A12b, four treated, Fig. A12f) during the study. Of the nine that produced flowers, only one tree (a treated tree at Olney SF) produced fruit (Fig. A12g), however the seeds were found to be non-viable. All flowers on the control trees showed signs of *A. psidii* infection.

Rhodomyrtus psidioides plots

In the *R. psidioides* plots, we identified 111 understorey species with only one Myrtaceae species, *Austromyrtus dulcis*, being present (in one plot) besides the *R. psidioides* seedlings (Table A11). We first tested whether fungicide treatment of the understorey affected species richness or abundance through time. We found no overall difference between control and treated plots (species richness $F_{3,84}$=0.88, p=0.45, Fig. 5a; total abundance $F_{4,102}$=0.57, p=0.68; Fig. 5b). Further, we found no

significant difference between treatments for richness ($F_{1,26}$=0.87, p=0.359) or total abundance ($F_{1,28}$=1.28, p=0.267) irrespective of the census time, although there appeared to be a trend of greater abundance in the treated plots at later census times (it must be remembered that abundance of *R. psidioides* seedlings was not included in this analysis). In contrast, there were significant differences between census time for both richness ($F_{3,84}$=4.62, p=0.005) and total abundance ($F_{4,102}$=3.91, p=0.005). As expected, the abundance of *R. psidioides* seedlings was significantly greater in the treated plots compared to the control plots at census times 4, 5 and 6 ($F_{4,101}$=5.83, p=0.001; Fig. 5c; see Fig. A12h for a picture of an infected seedling).

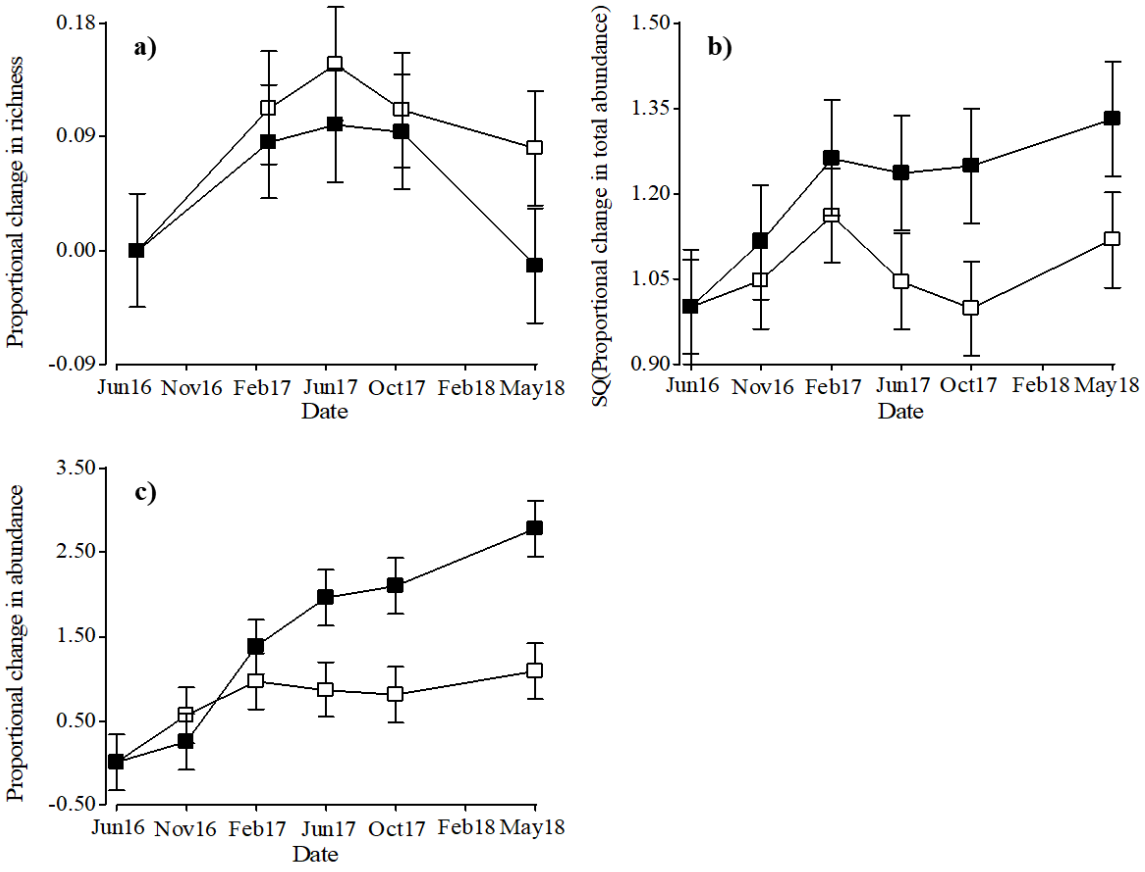

Fig 5 Proportional change through time for (**a)** understorey species richness, (**b)** understorey total abundance and (**c)** *Rhodomyrtus psidioides* seedling abundance for the *Rhodomyrtus psidioides* plots. The white squares represent the control plots while the black squares represent the treated plots. Vertical lines represent one standard error.

4. Discussion

To our knowledge, this study is the first to use the exclusion of a specific fungal pathogen to assess impacts beyond individual plant species to the community-level in the field. Previous studies have used an exclusion approach in the field to assess species-level effects or to assess general pathogen effects (see Bagchi et al. 2014). This study found that *A. psidii* directly and indirectly impacts on Australian rainforest vegetation at the community-level using fungicide spray treatments.

We found that untreated *R. rubescens* trees (infected with *A. psidii*) had on average 19% greater canopy transparency compared to the treated (uninfected) trees. This result supports the findings of Carnegie *et al*. (2016) that infection of *R. rubescens* by *A. psidii* results in substantial canopy dieback and leaf loss. However, Carnegie et al. (2016) reported from a three-year study of *R. rubescens* a more notable ~33% increase in canopy transparency, derived from the use of Schomaker et al.'s (2007) visual assessment technique. The discrepancy between this and our study in the magnitude of the canopy transparency increases may be due to the different canopy measuring techniques used, the greater duration of Carnegie's et al. (2016) study, or the fact that the infection of the trees was at a relatively earlier phase at the start of their study. Nevertheless, this range of increased canopy transparency is sufficient to reduce the photosynthetic leaf area, diminishing plants' ability to grow and reproduce, and in extreme cases result in mortality (Bamber and Humphreys 1965; Kulman 1971). In our study, we observed a modest 3% mortality rate in the control *R. rubescens* trees over the two-year study period while other

studies have reported marginally higher mortality rates for the same species (12% over 3 years in Carnegie et al. 2016; 5% over 2 years in Pegg et al. 2017).

Surprisingly, the increase in canopy transparency of the untreated *R. rubescens* trees did not translate into the predicted increase in the richness (8% decrease) and total abundance (46% decrease) of co-occurring understorey species in those plots. Rather, we found that canopy transparency was negatively associated with understorey species richness and total abundance. Although this appears counter-intuitive for rainforest vegetation, where canopy openings usually result in greater recruitment and growth of understorey species, it is possible that the denser canopies in the treated plots acted as nurse species for the seedlings of the understorey species (Niering et al. 1963; Gómez-Aparicio et al. 2004). That is, denser canopies protect vulnerable seedlings by shading them from heat as well as preventing soil moisture loss and photoinhibition (Powles and Thorne 1981; Powles 1984; Lovelock et al. 1994). Although our sites were located in rainforest communities which are normally defined by mild moist conditions (Adam 1992), this explanation is still relevant as during the summer months our sites regularly experienced days in excess of 35°C as well as below average rainfall for the period of the study (Bureau of Meteorology http://www.bom.gov.au/climate/current/annual/aus/). Furthermore, the germination of some shade tolerant understorey species has been shown to be triggered more readily by soil moisture availability than light availability (Augspurger 1979; Sork 1985). However, because we did not measure the difference in these environmental factors between control and treated plots we cannot confidently draw these conclusions.

Regarding the direct effects of *A*. *psidii* on *R*. *rubescens* understorey plots, results were as expected with increases of richness and total abundance in treated understorey plots. However, it is unlikely that this was due to Myrtaceae species thriving in absence of *A*. *psidii* infection, as no other Myrtaceae species were identified other than the study species. Rather, this direct effect may be explained by the fact that the fungicide used is a broad spectrum fungicide (not *A. psidii* specific), and therefore controlled other fungal pathogens that affect non-Myrtaceous taxa. It should be noted that we did observed the presence of other fungi in our plots, but did not identify them specifically.

For the *R. psidioides* plots, the richness and total abundance of co-occurring understorey species did not differ between fungicide-treated and untreated plots. This lack of difference may be due to *R. psidioides* trees being absent from the plots for some time prior to the study commencing so that any changes associated with *A. psidii* infection and canopy loss may have already occurred. If this is the case, then it is likely that in rainforest communities where *R. psidioides* was previously common, changes in their structure and function as a result of *A. psidii* may have already occurred. Previous studies have identified a number of communities which have experienced widespread *A. psidii*-associated mortality of *R*. *psidioides* trees (Pegg et al. 2014; Carnegie et al. 2016) where this may be the case. Another plausible explanation for the lack of difference between treatments could be the slow recruitment and growth of understorey species in rainforest communities (Denslow 1987; Lieberman et al. 1985). That is, our two-year experiment may not have been long enough to detect richness or abundance changes in the understorey species (excluding *R. psidioides*) present in our plots. However, this does not seem likely considering the understorey species in the *R. rubescens* plots did show differences in

that timeframe. On a side note, it was surprising that the recruitment of other Myrtaceae species was not detected in this study. This could be explained by a previous depletion of the seedbank caused by *A*. *psidii* infection, or a lack of disturbance promoting seed germination.

We found that the abundance of both *R*. *rubescens* and *R*. *psidioides* seedlings was significantly less (112% and 190% respectively) in the control understorey plots compared to treated understorey plots (it should be noted that proportional change is calculated based on abundance at census time 1 (which is not zero), therefore when final abundance is lower than the initial abundance, percentages are greater than 100%). This lower abundance was a result of both defoliation and mortality of seedlings for both study species, suggesting that recruitment of these susceptible species will be very limited which may contribute to their local extinction in areas where *A*. *psidii* occurs. Previously, Carnegie et al. (2016) reported limited seedling recruitment of the two study species across their native range (NSW and southeastern QLD), with all of them showing symptoms of *A*. *psidii* infection. Furthermore, Pegg et al. (2017) observed zero recruitment of *R*. *rubescens* seedlings at an affected QLD site. Both studies also found that the space previously occupied by the affected Myrtaceous species that experienced mortality tended to be filled by weed species such as *Lantana camara* (Carnegie et al. 2016; Pegg et al. 2017). Although no difference in species composition was found between treatments (data not presented), a similar trend was observed at our *R*. *psidioides* sites, with exotic species such as *Ochna serrulata, Ehrharta erecta* and *Stenotaphrum secundatum* having notably greater abundance in the control compared to fungicide treated plots. This is worrying as it suggests that *A. psidii*-related mortality of Myrtaceous species may facilitate the invasion of exotic species into native vegetation communities.

Therefore, it is important that long term monitoring and management efforts are made in *A. psidii*-affected areas to ensure that exotic species are not able to take advantage of space made available due to mortality of *A. psidii*-susceptible species. It should be noted that increases in the abundance of exotic species were not observed in our *R. rubescens* plots, most likely as the sites were not located in close proximity to urban areas so exotic propagule availability was low.

A particularly worrying finding of previous studies (Carnegie et al. 2016; Pegg et al. 2017; Pegg et al. 2018) was confirmed by our study - we found that *R. rubescens* trees irrespective of fungicide treatment produced none or very limited numbers of flowers and fruit, none of which produced viable seeds. This suggests that urgent conservation measures need to be taken to ensure the survival of this species into the future. The successful use of fungicide in controlling *A. psidii* in nurseries and forestry plantations suggests the possibility of targeted fungicide treatment as a potential management tool for *A. psidii*-susceptible species. However, this would be prohibitively costly in non-commercial environments such as natural vegetation, such that this is not a viable option (Ferreira 1983; Glen et al. 2007; Giblin 2013; *Chapter 2*). Thus in addition to seed and tissue collection for preservation, conservation efforts could focus on screening for *A. psidii* resistance genes using 'next generation' DNA sequencing techniques (Howard et al. 2015).

In the eight years since its detection in Australia, *A. psidii* has caused previously common Myrtaceae species to become critically endangered (NSW Scientific Committee 2017), as well as having pushed previously endangered species to the brink of extinction (Pegg et al. 2017; Makinson 2018a). With a host range of more than 370 species from one of the dominant plant families in Australia, *A. psidii*

poses a major risk to native Australian vegetation communities at a scale comparable to that of the soil borne pathogen *Phytophthora cinnamomi*. *Phytophthora cinnamomi* has been shown to have devastating effects on a number of Australian plant communities which in turn has had detrimental flow on effects to fauna that use those affected communities for habitat or food resources (Wills 1992; Weste 1994; Cahill et al. 2008). Similar to *A. psidii*, it has been listed as a threatening process in Australia, but *P. cinnamomi* has been present on the Australian continent for more than one hundred years (Weste 1994). This makes our finding that *A. psidii* was able to impact the structure and function of rainforest communities within a two-year period all the more worrying. Although the imminent extinction of critically endangered species as a result of *A. psidii* is likely (Carnegie et al. 2016; Pegg et al. 2018), it is critical that we understand the community-level flow on effects of these species losses. While *A. psidii* research in an Australian context is still in its infancy, the recently published 'Myrtle rust in Australia – a draft action plan' (Makinson 2018b) may help direct and stimulate further research to address critical questions about *A. psidii*, as well as build awareness of the potential devastating effects it may have if it remains unchecked. The outcome of these efforts will hopefully be the successful conservation of one of the most iconic plant families in Australia as well as the communities that they define.

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CHAPTER SIX

Discussion

Austropuccinia psidii is a globally invasive pathogen currently present in 27 countries across four continents (Carnegie and Pegg 2018). It was first detected in Australia in 2010 and has since spread from its introduction site in New South Wales (NSW) north to Queensland and Northern Territory and south to Victoria and Tasmania. This rapid spread was facilitated by long distance dispersing air-borne spores and accidental human transport (Carnegie and Lidbetter 2012; Pegg et al. 2014; Agriculture Victoria 2016). *Austropuccinia psidii* infects the new growth exclusively of species in the Myrtaceae family producing impacts at the individual-level (leaf necrosis and distortion, defoliation, branch dieback, reduced reproductive output and mortality) (Rayachhetry et al. 2001; Uchida et al. 2006), species-level [(e.g. widespread mortality of *Rhodamnia rubescens* and *Rhodomyrtus psidioides* (Carnegie et al. 2016; Pegg et al. 2018a)] and potentially at the ecological community-level. Although some fungal pathogens are known to have had substantial impacts on species with subsequent flow-on community-level effects [(e.g. Dutch elm disease, chestnut blight, white pine rust (Maloy 1997; Brasier 2000; Elliot and Swank 2008)], very little is known about the potential impacts of *A*. *psidii* on Australian vegetation communities.

Australian vegetation may be particularly susceptible to large-scale impacts as many are dominated by Myrtaceae and have high concentrations of new growth after fire, a common natural disturbance (Gill 1975). Therefore, the overarching goal of this thesis was to determine the impacts of the invasive pathogen, *Austropuccinia psidii*, on Australian native vegetation communities. To do this, several approaches were used including a survey of natural resource managers, glasshouse experiments to test susceptibility of species to *A*. *psidii* as well as species-level impacts after fire, and a field experiment to test the cascading community-level impacts.

In *Chapter 2* I described the outcomes from a survey distributed to researchers, land managers and government employees that aimed to better understand the geographic extent and impacts of *A*. *psidii* on Australian native vegetation communities. The responses to this survey provided valuable up-to-date information on the occurrence of *A*. *psidii* and susceptible host species within the current range of *A*. *psidii*, which will assist in management decisions for the conservation and restoration of affected areas. Furthermore, the survey helped identify some key knowledge gaps that I was able to address in this thesis. Firstly, although the survey provided some new information on susceptibility of host species, more than half of the native Australian Myrtaceae species, including a number of endangered species, still lacked a susceptibility status. Therefore, the aim of *Chapter 3* was to test the susceptibility of 24 previously untested species/sub-species, with a particular emphasis on endangered species. The second key knowledge gap I addressed in this thesis was to quantify the impact of *A*. *psidii* on plant architecture, growth and biomass allocation after fire (*Chapter 4*). Given that fire is a frequent disturbance in Australia and new growth from fire-protected buds in resprouting species is highly susceptible to *A*. *psidii* infection, it was clear that testing this interaction should be a priority. Finally, I assessed the impacts of *A*. *psidii* on Australian native rainforest communities in situ, through a large scale field experiment. More specifically, I investigated both indirect and direct impacts of *A*. *psidii* invasion on community species richness and abundance (*Chapter 5*). The key results for each chapter is summarised below, with ongoing and future management steps as well as key research gaps also discussed.

Key results

Increasing knowledge on current distribution of A. psidii in Australia

In *Chapter 2* of this thesis, four new local government areas (LGAs) were identified by survey respondents where *A*. *psidii* had not been previously recorded. The LGAs are located in the two states where *A*. *psidii* is currently most widespread (NSW and QLD), confirming the mainly east coast distribution of the pathogen and where monitoring for *A. psidii*, although limited, has been concentrated (Carnegie et al. 2016; Pegg et al. 2017). This result highlights the value of the natural resource managers' knowledge especially in the absence of a national monitoring program. In Victoria and Tasmania, *A. psidii* has only been recorded in urban gardens, parks and nurseries but fortunately not in the natural environment, unlike the Northern Territory where it has been detected in native vegetation communities (Westaway 2016; Makinson 2018a; Westaway 2018). Furthermore, it is yet to be detected in South and Western Australia. To prevent the spread of *A. psidii* to these unaffected states, it is essential that we continue to track its spread in the states where it is currently present as well as maintain strict quarantine biosecurity measures on Myrtaceae importations while raising public awareness on the impacts of the pathogen. For example, Victoria has implemented a sentinel tree program to assist in monitoring the spread of *A*. *psidii* (D. Smith, Agriculture Victoria, pers. comm.). These practices can also be useful in a broader context as it is vital that we avoid the introduction of other *A. psidii* biotypes into Australia from around the world.

Increasing knowledge on host susceptibility in Australia

At the commencement of this study, *A*. *psidii* was known to have 347 susceptible hosts among Australian Myrtaceae species. However, it was believed that many more Myrtaceous species were potentially susceptible. As a result of data collected in this study, a further 21 susceptible species (20 native) and one genus have been identified that were previously not included in the most recent national host species lists of Berthon et al. (2018) or Makinson (2018a). Four of these species were identified as hosts of *A*. *psidii* through the responses of survey participants (*Chapter 2*), while the remaining 17 (12 of which are listed as endangered under state or national legislation), were identified from susceptibility tests outlined in *Chapter 3*. These results increase the number of known susceptible hosts in Australia to 378 native species in 50 genera.

Austropuccinia psidii is known to infect 539 Myrtaceae species in 79 genera worldwide (Giblin and Carnegie 2014; Makinson 2018a; Soewarto et al. 2018; *Chapter 2* and *3*), with more than 65% of the known hosts native to Australia. It is worthwhile mentioning that in the literature, depending on the taxonomic criteria used, different numbers of susceptible species and genera are reported for Australia. For example, Carnegie and Pegg (2018) state that there are 347 native susceptible species in 57 genera, while Berthon et al. (2018) list 380 susceptible species in 52 genera. However, the latter study contains ~19 species flagged as doubtful records by Makinson (2018b), as they include for example species that did not show a capacity to sustain the complete fungal life cycle when tested under controlled conditions. For this thesis, we have adopted the most recent figures put forward by

Makinson (2018a) of 358 susceptible native species/sub-species in 49 genera, where taxonomic names are based on the Australian plant census.

In addition to identifying new susceptible host species, *Chapter 3* of this thesis has also contributed to the growing literature that shows inter- and intra-specific variability in species susceptibility to *A. psidii* tested in Australia and Brazil (e.g. Ferreira 1983; Dianese et al. 1984; Zauza et al. 2010; Morin et al. 2012; Sandhu and Park 2013; Potts et al. 2016). These between and within species differences suggest the presence of resistance genes, which provides hope to conservation efforts particularly in relation to endangered species. The challenge is that selecting for resistance genes is onerous, time consuming and expensive. For these reasons, this approach has potential for commercial species (e.g. *E. grandis*), but may not be a viable option for conservation and restoration purposes (Makinson 2018a). However, resistance genes may spread naturally through populations by means of natural selection, provided that the impacts of *A. psidii* are slow enough to allow for this to occur (Makinson 2018a). It should be noted that caution must be taken when translating controlled glasshouse susceptibility results to the field, as different susceptibility scores in the glasshouse compared with field studies have been reported for the same species, even when using a consistent scoring method (Pegg et al. 2018a). The cause of these differences is not well understood, but it has been suggested that varying environmental conditions between the two may be responsible. Therefore, it is important to corroborate controlled glasshouse susceptibility results as well as impacts with field observations.

Understanding the interaction between fire and A. psidii

In addition to Australia having the majority of the world's susceptible host species and a large climatically suitable area for *A. psidii* (Berthon et al. 2018), the impacts of *A. psidii* may be further exacerbated by fire which is a frequent disturbance in many Australian vegetation communities. This is because plants resprouting after fire have a flush of new growth that will be susceptible to infection (Grgurinovic et al. 2006; Glen et al. 2007). The large number of resprouting and regenerating plants within a small time period at a particular location may facilitate the pathogen and make infection more likely or more severe. Despite this likely interaction between fire and *A. psidii*, only one previous study has attempted to assess it (Pegg et al. 2018a). This study assessed the impacts of *A*. *psidii* on nine Myrtaceae species after a natural fire in the field, and reported that after repeated infections the seedlings lost apical dominance and became multi-branched (Pegg et al. 2018a). However, the lack of a disease exclusion treatment did not allow for definite conclusions to be made on the impact of the pathogen. Therefore, gaining a better understanding of the impacts of *A*. *psidii* after fire represents a crucial area of research.

This thesis addressed this by assessing the impacts of *A*. *psidii* on eight known susceptible host species after fire in controlled glasshouse conditions (*Chapter 4*). Similarly to Pegg et al. (2018a), we found that post-fire infected plants were shorter but more highly branched compared to uninfected plants. This could result in less susceptible species, or non-Myrtaceae, out-competing susceptible species for natural resources and subsequently dominating fire-prone sites. We also found that infected resprouting individuals had reduced specific leaf area as expected, but increased total leaf biomass despite defoliation, which was contrary to expectations. Similarly,

Carnegie et al. (2016) detected reduced leaf area in *R*. *rubescens* in trees infected with *A. psidii* compared to un-infected trees in a disease exclusion field experiment. It should be noted that although the experiment described in *Chapter 4* showed that infected individuals had modified architecture and growth after fire, this experiment was relatively short term (38 weeks) because of time constraints. A longer experiment incorporating multiple inoculation events is recommended to elucidate the likely impact of *A. psidii* infection in post-fire vegetation. In addition, field based studies are required to confirm the impact of *A*. *psidii* on fire prone communities and the potential related changes in community structure suggested by the results from glasshouse studies.

Understanding the ecological impacts of A. psidii

Given that previous studies as well as the earlier chapters of this thesis have focussed mainly on species-level impacts of *A*. *psidii*, our understanding of the community-level impacts is rudimentary. *Chapter 5* of this thesis describes a large multi-year experiment that assessed the direct and indirect impacts of *A. psidii* on temperate rainforest communities of New South Wales, Australia, using the highly susceptible host species *R. rubescens* and *R. psidioides* as focal species. The experiment was fully factorial with the treatments being exclusion of *A. psidii* from canopy host plants and exclusion from understorey vegetation, with exclusion being achieved through fungicide application. This allowed us to test the direct and indirect effects of *A. psidii*. *Rhodamnia rubescens* trees that were not treated with fungicide had significantly greater canopy transparency than sprayed trees. We expected that increased canopy transparency would subsequently promote seedling recruitment, resulting in

increased abundance and species richness of the understorey vegetation, including potential exotic species invasion if propagules were present in the area. Surprisingly, increased canopy transparency was associated with a decrease in the richness and total abundance of co-occurring understorey species. Possible explanations for these results are that understorey species were negatively affected by photoinhibition (Lovelock 1994) or reduced soil moisture availability when canopy transparency increased, rather than taking advantage of the increased light availability. This suggests that the canopy of *R. rubescens* trees could be acting as nurse species (Niering et al. 1963) providing the conditions that facilitate the understorey species persistence. Another plausible explanation is that the increased light availability promotes the invasion of exotic species causing subsequent reductions in diversity, as shown by the invasion of *Lantana camara* in disturbed wet sclerophyll forest (Cummings et al. 2007; Gooden et al. 2009).

For fungicide treated *R. rubescens* canopies, we expected that fungicide treatment of the understorey would enable understorey Myrtaceous species to survive, resulting in greater richness and abundance in fungicide treated understorey plots compared to the control plots. Although we did not find differences between treatments for species richness, total abundance increased in fungicide treated plots as expected. This result suggests a direct effect of the broad spectrum fungicide on species abundance, which could be controlling for other fungal pathogens concurrently. For the *R. psidioides* plots, no difference for richness and total abundance existed between the sprayed and control understorey plots. However, as expected, the abundance of seedlings (recruitment) of the two study species increased in the fungicide spray treatment. Finally, we found very scarce flower, fruit and seed production in *R. rubescens* trees irrespective of fungicide treatment. These

findings as well as those of previous studies suggest that a significant reduction in both the recruitment and survival of the focal species *R. rubescens* and *R. psidioides* is likely in the long term (Carnegie et al. 2016; Pegg et al. 2018a). On a side note, an interesting observation we made was that the recruitment of Myrtaceae species across all of our sites was almost non-existent. This suggests that prior to the start of the experiment, *A. psidii* may have already depleted the seedbank of the Myrtaceae species by causing mortality in newly recruited seedlings.

Austropuccinia psidii: a broad perspective

This thesis confirmed that *A. psidii* has a widespread distribution in Australia, and models suggest that climate suitability will continue to be conducive for the pathogen in the future (Berthon et al. 2018). The distribution of *A. psidii* as well as its host species tend to be located in fire-prone woodland vegetation communities, therefore interactions between *A. psidii* and fire are to be expected, with highly susceptible species being particularly vulnerable to this threat. This is because plants tend to be more vulnerable to pathogens after fire, which in the case of *A. psidii* would be due to the higher proportion of vulnerable new growth in resprouting plants. Therefore, it is likely that after multiple fire and re-infection events, plants will eventually run out of carbohydrate reserves to resprout, resulting in mortality (Carnegie et al. 2016). In our experiment, two of the *R. rubescens* adult trees died as a result of *A. psidii* infection. At a species-level, this mortality could eventually lead to the extinction of species, at least locally. At a broader scale, this mortality may alter fire regimes at the community-level. That is, the widespread mortality of highly susceptible species may increase the fuel load within the community thus increasing fire frequency. This

could potentially cause a feedback loop between re-infection and mortality until these highly susceptible species drop out of the community completely (Carnegie et al. 2016).

In addition to the rainforest communities that this thesis has focused on, impacts in other vegetation communities have been reported including in coastal heath, wet and dry sclerophyll and wetlands as well as threatened ecological communities under the Commonwealth *Environment Protection and Biodiversity Conservation Act* (e.g. Blue Gum High Forest of the Sydney Basin Bioregion) (Pegg et al. 2014, 2017; Makinson 2018a; Pegg et al. 2018a; *Chapter 2*). All these communities are dominated by Myrtaceae species or have a significant Myrtaceous component. For example, wetland communities can be formed by monospecific stands of *Melaleuca* spp. (broad-leaved paperbark trees) including *M*. *quinquenervia*, *M*. *leucadendra* and *M*. *viridifolia* (Cook et al. 2008). These are known as keystone species, as they provide a wide range of ecological services including maintenance of water temperature, improvement of water quality and provision of habitat and food for fauna species (Lepschi 1993; Eby 1995). These three species are all susceptible to *A*. *psidii* to varying degrees, ranging from relatively tolerant individuals to highly susceptible (Pegg et al. 2018b). Although 25-41% of individuals in these species have been shown to be resistant (Pegg et al. 2018b), it is still likely that significant flow-on community-level impacts will result from the potential widespread mortality of highly susceptible individuals before natural selection can act.

The already large number of *A. psidii* native host species in Australia has increased to 378 species as a result of this thesis (Makinson 2018a; *Chapter 2* and *3*). These host species are infected by the pandemic biotype alone (Machado et al. 2015),

which is different from the biotypes present for example in Brazil or South Africa (Stewart et al. 2018). Climate models suggest that the former biotypes could thrive in Australian climatic conditions (Stewart et al. 2018). If these biotypes arrive in Australia [there are at least three others (Ross-Davis 2014)], the number of susceptible hosts could increase significantly, the impacts on existing hosts could become more severe (MacLachlan 1938; Rayachhetry et al. 1997) and the geographical distribution of the pathogen could increase (Makinson 2018a). New biotypes would represent a significant threat not only to the native vegetation communities, but also to commercial plantations (e.g. *Eucalyptus grandis* and *Backhousia citriodora* (lemon myrtle)), orchards and plant nurseries (Cannon 2011; Berthon et al. 2018; Carnegie and Pegg 2018; Makinson 2018a; Stewart et al. 2018).

Studies of *A. psidii* impacts in other countries are scarce and usually focus on commercial forest plantations rather than native communities (Carnegie and Pegg 2018). For example, *A. psidii* has caused significant damage in commercial plantations (exotic *Eucalyptus grandis*) and orchards (native *Psidium guajava*) in South and Central America (Tommerup et al. 2003; Ribeiro and Pommer 2004). While field surveys have been conducted in countries including the United States, South Africa, New Caledonia and New Zealand to detect infected species in the natural environment (Loope 2010; Roux et al. 2016, Herrera 2018; Soewarto et al. 2018), none of these studies have assessed community-level impacts. Despite the lack of *A. psidii* studies on community-level impacts, we can learn lessons from the previous invasions of other plant fungal pathogens across the world. There are many examples of invasive plant fungal pathogens having severe impacts on native vegetation communities in North America and Europe (Ellison et al. 2005; Loo 2009; Budde et al. 2016). These include chestnut blight (*Cryphonectria parasitica*)

(Anagnostakis 1987; Elliot and Swank 2008), Dutch elm disease (*Ophiostoma ulmi*) (Gibbs 1978; Brasier 2000), sudden oak death (*Phytophthora ramorum*) (Rizzo et al. 2005), ash dieback (*Hymenoscyphus fraxineus*) (Pautasso et al. 2013) and white pine blister rust (*Cronartium ribicola*) (Maloy 1997). Similarly to *A. psidii* with *R. rubescens* and *R. psidioides* in Australia (Carnegie et al 2016, Pegg et al. 2018a, *Chapter 5*), all these pathogens cause widespread host tree mortality. For example, chestnut blight has virtually eliminated adult *Castanea dentata* trees from North America (Budde et al. 2016). In an Australian context, a similarly devastating exotic plant pathogen invasion, *Phytophthora cinnamomi,* has occurred and continues to impact native vegetation, particularly in Western Australia (Burgess et al. 2017). This pathogen has a wide range of host species which when affected causes dramatic decreases in species richness and abundance as well as cascade effects on associated fauna (Cahill et al. 2008; Commonwealth of Australia 2014). Given a greater residency time it is not unreasonable to suggest that *A. psidii* may have similar impacts to *P. cinnamomi* in the longer term. Like *P. cinnamomi,* eradication of *A. psidii* is not feasible in the states where it is currently established (NSW, QLD and NT), and we have to accept that species extinctions in the wild are likely to occur (Carnegie et al. 2016; Makinson 2018a; Pegg et al. 2018a). However, there are management steps that can be undertaken to limit the impact it has on Australian vegetation communities as a whole. A number of key ongoing and future management steps are discussed below.

Ongoing and future management steps

Once established, plant fungal pathogens can be impractical to eradicate (Makinson 2018a). Therefore, introduction prevention or at the very least early detection are

the only feasible options to avoid the negative impacts associated with plant fungal pathogen invasions (Santini et al. 2013; Roy et al. 2014). Considering this in an Australian context, it is vital that at the national level the current biotype present in eastern Australia is prevented from reaching South Australia and Western Australia. Western Australia is particularly vulnerable as it is a biodiversity hotspot with a large number of endemic plant species (Brooks et al. 2002). At the global scale, it is also important to prevent different biotypes spreading between different countries (Loope and La Rosa 2008) as different species are known to be vulnerable to different biotypes (MacLachlan 1938; Rayachetry et al. 1997). This is particularly relevant to Australia as it has the majority of the world's species susceptible to the pandemic biotype (Machado et al. 2015). It is likely that the number of susceptible host species would increase if other *A*. *psidii* biotypes were present on the continent.

For species and communities that are already affected, salvaging positive outcomes from a bad situation should be the priority. The vital first step in obtaining positive outcomes has been to consolidate the highly fragmented *A. psidii* data (as highlighted in *Chapter 2*) into an easy to understand and readily available source. Achieving this will facilitate government agencies and the wider community to become more engaged in the issue. As Jacobs et al. (2013) pointed out, it is vital for the social 'sphere' to be incorporated with the ecological 'sphere' in order for restoration and conservation goals to be achieved. The recent publication of the Draft Management Plan (Makinson 2018b) will hopefully trigger funding and momentum on myrtle rust research, as well as lead to national level coordination in maintaining up-to-date information on hosts, impacts and distribution of *A*. *psidii* in Australia. The information obtained through this thesis has contributed significantly to the latter in

particular by updating the national host species list which will help in prioritising species (e.g. 12 susceptible endangered species reported in *Chapter 3*) and regions for future management.

Although many Myrtaceae have been shown to be susceptible to *A. psidii* in controlled glasshouse conditions (*Chapter 3*), it has been highlighted in the Draft Management Plan that corroborating these susceptibility tests with field observations should be of the highest priority. Threatened species that are yet to be assessed in the field, including the ones tested as part of *Chapter 3*, are the obvious candidates to be assessed first, with a particular emphasis placed on the 12 species that have been pushed to the brink of extinction by *A. psidii* (Pegg et al. 2014, Carnegie et al. 2016; Pegg et al. 2017; *Chapter 5*). Coupled with these field observations should be ongoing germplasm collection (in the form of either seeds or tissues) which is crucial as it will allow the genetic rescue of endangered species as well as enable research into identifying resistance genes (Makinson 2018b). In addition, identification of less susceptible plants for future propagation and translocation action is crucial.

Research gaps

As discussed above, *A. psidii* research in an Australian context is still in its infancy and many research gaps still exist. Many of these have been identified and outlined in the Draft Management Plan (Makinson 2018b) and have been given a priority rating, albeit no guaranteed funding. Therefore, I briefly discuss two key novel research gaps that are of particular interest to me and that I believe warrant research attention. Firstly, studying the impacts of *A*. *psidii* on species that are of high value to Australian

aboriginal people may prove fruitful in terms of preserving indigenous Australian culture. This socio-ecological dimension was successfully considered during the recent invasion of *A*. *psidii* in New Zealand, where Māori people were consulted during the emergency government response and subsequent development of a long-term management plan (MPI 2018). The second research gap is an extension of *Chapter 5* and relates to the cascading effects of *A*. *psidii* on fauna which rely on *A. psidii* susceptible host species as a food resource or shelter within affected plant communities (Carnegie et al. 2016; Makinson 2018a; Pegg et al. 2018a). As discussed above, these flow-on effects on fauna are likely, as has been the case for other fungal pathogens such as *P. cinnamomi* (Wills 1993; Ayres and Lombardero 2000). Cascading effects that could be studied include the infection of fruits that are a dietary component of co-occurring birds and mammals, the loss of canopy affecting the nesting behaviour of fauna species and the impacts of yellow *A*. *psidii* urediniospores confusing bees while foraging (Chapman 1964; Pattemore et al. 2018). It has already been suggested that the previously observed insect pollinator and bird visitations to *R*. *psidioides* trees for flowers (Williams 2018) and fruit (Church 1997) in the 1990s might no longer being seen as a result of the arrival of *A. psidii*.

It has been eight years since *A*. *psidii* was first detected in Australia, triggering an emergency government response. In the time since that initial detection, no long term environmental management plan had been put in place for this Key Threatening Process [EPBC Act 1999: Novel biota and their impact on biodiversity (2013)]. However in May 2018, with the valuable input of the 'Myrtle rust environmental impacts working group', a Draft Action Plan (Makinson 2018b) was published to set the foundation for a nationally coordinated strategy aimed at the conservation of

Australia's iconic native biodiversity. Despite this positive step, it is clear that much work still needs to be done for us to truly grasp the potential impacts of the *A. psidii* invasion in Australia. This thesis represents a significant contribution to this body of literature and is an important first step in understanding the overall picture of the *A. psidii* invasion within Australia.

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APPENDICES

Appendix 1 Chapter 2

A1 Survey sent to participants on December 2015 and closed on February 2016.

Date of entry: / /

MYRTLE RUST SURVEY

We are undertaking a study assessing the ecological impacts of Myrtle rust in Australian native plant communities as well as on forestry species. This survey is being carried out by Laura Fernandez, a PhD student from Macquarie University (Sydney). Your participation is very important to increase our understanding of this invasive fungal pathogen. We thank you very much for taking the time to answer this survey, it will only take you between 5-10 minutes. Your responses will be confidential and anonymous. A summary of the information gathered here will be sent to you on completion of the project. Please if possible reply by 5th February 2016. If you have any questions please send an email to laura.fernandez@students.mq.edu.au

Please reply YES or NO by circling your selected answer, complete the tables or write down your answers when needed.

1. Have you seen evidence of Myrtle rust infection on plants in your area? (Example pictures of infected plants, fungus yellow bright spores are shown below).

a) YES (continue) b) NO (go straight to question 2)

a) At what localities have you observed Myrtle rust?

Locality name (e.g. Royal National Park, Olney State Forest)	**Locality Type** (e.g. State Forest, conservation area, garden)	**Local Government Area**

b) If you know the plant community type please specify (e.g. coastal woodland, heath, grassy woodland, tall open forest, rainforest, etc.).

_____

_____

_____

c) Can you remember which plant species you have seen infected? If yes, please complete the table below.

Species infected	**Level of infection** (low, medium or high)	**Tissues attacked** (e.g. leaves, flowers, fruits)	**Defoliation present**	**Plant mortality observed**

d) If you have Myrtle rust in the area you manage, are you trying to control it? If yes, please explain what control or containment measures you are using. If no, please outline why.

_____

_____

_____

e) Do you think Myrtle rust represents a long-term threat to any plant communities or species? If yes, please describe how.

_____

_____

_____

Please continue on to answer questions 2a, 3 and 4.

2. Myrtle rust is an invasive fungus native to South America that arrived in Australia in 2010 and attacks plant species within the Myrtaceae family. Known susceptible species include eucalypts, bottlebrush, paper-bark and lemon myrtle, causing defoliation, multi branched growth and sometimes death of very susceptible individuals. You can go to https://www.anbg.gov.au/anpc/images/Puccinia%20psidii%20Australia%20Hostlist%2024Sept2014%20WORDtable.pdf for a list of Australian host species.

a) Have you received information about this invasive fungus from Government agencies such as NSW Department of Primary Industries?

_____

_____

_____

b) Do you work for local government, state government, or private consulting company? Please specify.

_____

_____

3. Are you willing to be contacted about this issue and receive more information? (Yes/No). If yes, please provide contact details such as an email address.

_____

_____

4. Are there any comments or observations you would like to add?

_____

_____

_____

Thank you so much for your time!

Appendix 2 of this thesis has been removed as it may contain sensitive/confidential content

A3 Different terminologies used by participants regarding plant community types and grouped categories are detailed:

Natural:

"**Rainforest**" = rainforest + littoral rainforest + moist hardwood forest types + lowlands sub-tropical rainforest

"**Tall open forest**" = Tall open forest + wet sclerophyll forest

"**Coastal woodland**" = coastal woodland + coastal wetland

"**Riparian/gully**" = riparian + creek + riverbanks + wet sclerophyll gully

Artificial:

"**Garden**" = garden + ornamental + botanical gardens

"**Street tree**" = street trees + planted + plantings

"**Park**" = park + Municipal parkland

Natural	
Rainforest	46
Tall open forest	22
Coastal woodland	9
Riparian / gully	6
Swamp sclerophyll forest	5
Dry sclerophyll forest	1
Closed forest	1

Artificial	
Garden	38
Street tree	12
Plant nursery	12
Botanical gardens	6
Park	4

Table A4 Level of infection classes defined according to the frequency with which *A*. *psidii* affects different tissues and leads to plants mortality.

	Low	Medium	High
Leaves	Yes	Yes	Yes
Stems	Rarely	Yes, sometimes	Yes, often
Flowers	Rarely	Yes, sometimes	Yes, often
Fruits	Rarely	Yes, sometimes	Yes, often
Mortality	No	Yes, sometimes	Yes, often

Table A5 Local government areas (LGAs) by Australian state or territory where *Austropuccinia psidii* has been observed or not observed in this study. Number (No.) represents number of times that *A. psidii* has been observed by individual respondents in the relevant LGA in this study. New LGAs for which *A*. *psidii* has not been previously reported are represented in bold. Note that LGAs not mentioned in this survey are not present in this table.

State	LGAs observed	No.	LGAs not observed	No.
ACT			Canberra	0
NSW	Ballina	1	Bathurst Regional	0
	Bankstown	1	Blacktown	0
	Blacktown	1	Bland	0
	Blue Mountains	1	Blue Mountains	0
	Byron	4	Camden	0
	Camden	1	Corowa Shire	0
	Clarence Valley	2	Dubbo	0
	Coffs Harbour	3	Gwydir	0
	Eurobodalla	1	Hawkesbury	0
	Gosford	2	Lane Cove	0
	Great Lakes	6	North Sydney	0
	Greater Taree	3	Penrith	0
	Kempsey	3	Singleton	0
	Ku-ring-gai	2	Sydney	0
	Lake Macquarie	4	Tumut Shire	0
	Lane Cove	1	Waverley	0
	Leichardt	1	Wollondilly Shire	0
	Lismore	5	Woollahra	0
	Marrickville LGA	2		
	Muswellbrook	1		
	Nambucca	2		
	Newcastle	3		
	Parramatta	2		

	Pittwater	1		
	Port Macquarie - Hastings	9		
	Port Stephens	1		
	Richmond Valley	1		
	Ryde	1		
	Shellharbour	1		
	Shoalhaven	3		
	Singleton	1		
	Strathfield	2		
	Sutherland	2		
	The Hills Shire	1		
	Townsville	1		
	Tweed	3		
	Warringah	1		
	Willoughby	1		
	Wollongong	3		
	Wyong	5		
NT			Darwin	0
QLD	Brisbane	1	Charters Towers	0
	Burdekin	1	Kowanyama	0
	Cairns	1	North Burnett	0
	Gladstone	1	Western Downs	0
	Logan	1		
	Mackay	1		
	Redland	1		
	Scenic Rim	1		
	Sunshine Coast	3		
TAS			Circular Head	0
			Devonport	0
			Glamorgan/Spring Bay	0
			Glenorchy	0
			Hobart	0
			Huon Valley	0
			Northern Midlands	0
VIC	Melbourne	1	Banyule	0
	Monash	1	Baw Baw	0
	Stonnington	1	Benalla	0
	Surf Coast	1	Boroondara	0
	Yarra	1	East Gippsland	0
			Greater Bendigo	0
			Greater Dandenong	0
			Greater Geelong	0
			Latrobe	0
			Mitchell Shire	0

Monash	0
Mornington Peninsula	0
Murrindindi	0
Southern Grampians	0
Surf Coast	0
Whitehorse	0

Note: ('not observed' means that one or more participants recorded that *A*. *psidii* had not been observed in that particular LGA by them; it does not necessarily mean that it is absent from that area).

Appendix 2 Chapter 3

Table A5 Area of occurrence of each study species, and current and future percentage overlap between the study species and *Austropuccinia psidii*. Even though this criterion was implemented for four species only (in bold), we present values for all study species.

Species	Area of occurrence	Overlap curr. %	Overlap fut. %
Callistemon megalongensis	3	0	0
Eucalyptus amplifolia	314	51	53
Eucalyptus boliviana	5	0	0
Eucalyptus castrensis	3	0	0
Eucalyptus copulans	5	0	100
Eucalyptus deuaensis	8	100	100
Eucalyptus fracta	5	40	60
Eucalyptus largeana	41	88	90
Eucalyptus macarthurii	60	40	52
Eucalyptus magnificata	29	0	3
Eucalytpus ophitica	9	100	100
Eucalyptus pachycalyx	99	39	22
Eucalyptus parvula	61	2	3
Eucalyptus scoparia	32	6	6
Eucalyptus scopulorum	2	0	0
Kardomia prominens	3	100	100
Leptospermum petraeum	8	100	0
Melaleuca irbyana	69	97	91
Melaleuca tortifolia	7	100	100
Micromyrtus blakelyi	17	94	100
Triplarina imbricata	15	93	100
Triplarina nowraensis	17	100	100

Table A6 Provenance of study species and sub-species tested for susceptibility (specific locations are not provided for endangered species). All provenances in this table belong to New South Wales.

Species	Provenance
Eucalyptus amplifolia amplifolia	Sawyers Reserve
Eucalyptus amplifolia amplifolia	Sun Valley
Eucalyptus amplifolia sessiliflora	Tenterfield
Eucalyptus boliviana	Bolivia Hill Nature Reserve
Eucalyptus deuaensis	Mongamulla Mtn.
Eucalyptus fracta	Pokolbin State Forest
Eucalyptus ophitica	Copmanhurst
Eucalyptus scopulorum	Peregrine Point Picnic Area
Kardomia prominens	Nymboida NP
Leptospermum petraeum	Kanangra Walls
Melaleuca tortifolia	Barren Mountain
Micromyrtus blakelyi	South Maroota
Triplarina imbricata	Drake
Triplarina nowraensis	Nowra

Fig A7 Images of different aspects of the experiment: **a)** general view of the glasshouse with individual plants in pots, **b)** *A*. *psidii* urediniospores suspended in mineral oil previous to inoculation, **c)** the positive control *S. jambos* showing high susceptibility and therefore viability of the inoculum, **d)** *E*. *boliviana* with no signs of infection (resistant), **e)** *E. castrensis* with two yellow pustules indicated by red arrows, and **f)** *E. copulans* with several pustules on different leaves (highly susceptible).

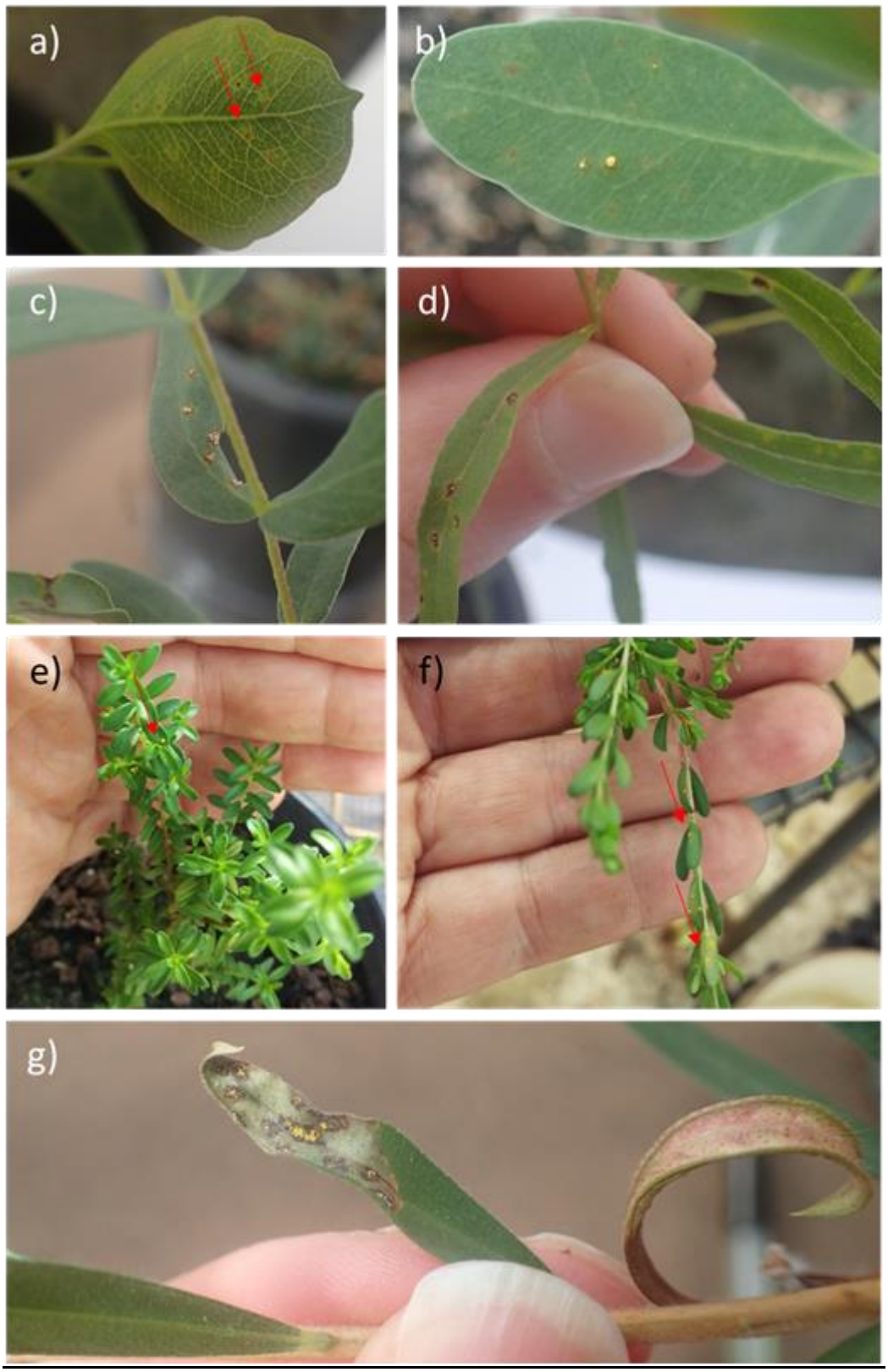

Fig A8 Symptoms of *A. psidii* infection (yellow pustules) on individuals of **a)** *E. magnificata*, **b)** *E. pachycalyx pachycalyx*, **c)** *E. parvula*, **d)** *E. scoparia*, **e)** *Kardomia prominens*, **f)** *Triplarina nowraensis* and **g)** *Melaleuca megalongensis.*

Appendix 3 Chapter 4

Table A9 The known susceptibility scores of the original 12 study species according to the most updated list in Berthon et al. (2018), with the following susceptibility scale: R=Resistant, L=Low susceptibility, M=Medium susceptibility, H=High, S=Susceptible but severity not recorded; followed by susceptibility results from this study according to Pegg et al. (2014), and then translated to Berthon et al. (2018) scale for comparison. Updated severity records can be found in bold. Only the eight species that had enough resprouting individuals (≥5) after being burnt were included in the data analyses.

Species	Known suscept.[6]	Suscept. results[5]	Suscept. results[6]	N
Angophora costata	R-H[1]	M-H	M-H	9
Callistemon citrinus	R-H[1,2]	R-M	M	10
Callistemon rigidus	S[3]	**R-H**	**M-H**	10
Eucalyptus dalrympleana	R-H[4]	R-M	M	5
Eucalyptus globoidea	H[2]	**M-H**	**M-H**	6
Eucalyptus moluccana	R-H[1,2]	R-M	M	10
Eucalyptus sieberi	R-H[4]	M	M	1
Eucalyptus viminalis	S[3]	**M-H**	**M-H**	6
Leptospermum juniperinum	S[3]	-	-	-
Melaleuca nodosa	H[5]	M	M	2
Melaleuca sieberi	S[3]	**H**	**H**	2
Melaleuca styphelioides	S[3]	**R-M**	**M**	10

[1](Morin et al. 2012); [2](Sandhu and Park 2013); [3](Giblin and Carnegie 2014); [4](Potts et al. 2016); [5](Pegg et al. 2014); [6](Berthon et al. 2018)

Fig A10 Images of different aspects of the experiment **a)** plants growing previous to being burnt, **b)** plants after the fire treatment, **c)** individuals resprouting; and inoculated plants showing signs of infection (yellow pustules and/or necrosis) **d)** *Angophora costata*, **e)** *Callistemon citrinus*, **f)** *C. rigidus*, **g)** *Eucalyptus dalrympleana*, **h)** *E. globoidea*, **i)** *E. moluccana*, **j)** *E. viminalis*, and **k)** *Melaleuca styphelioides*.

Appendix 4 Chapter 5

Table A11 List of taxa identified to the family, genus or species level in *Rhodamnia rubescens* (93) and *Rhodomyrtus psidioides* (77) plots at Royal National Park, Wambina Nature Reserve and Olney State Forest for the former, and Wamberal Lagoon Nature Reserve, Shelly Beach and Bundjalung National Park for the latter.

Species *R. rubescens* plots	Species *R. psidioides* plots
Acacia disparrima	*Acacia disparrima*
Acacia madenii	*Acacia spp.*
Adiantum spp.	*Alchornea ilicifolia*
Alchornea ilicifolia	*Anredera cordifolia*
Asteraceae	*Araujia sericifera*
Banksia integrifolia	*Asparagus aethiopicus*
Banksia spp. 2	*Asparragus plumosus*
Blechnum cartilagineum	*Asteraceae (exotic)*
Breynia oblongifolia	*Asteraceae (native)*
Calochlaena dubia	*Austromyrtus dulcis*
Carex brunnea	*Baloghia inophylla*
Casuarina spp.	*Banksia integrifolia*
Cayratia clematidea	*Bidens pilosa*
Centella asiatica	*Breynia oblongifolia*
Cinnamomum camphora	*Canoza bonariensis*
Cissus antarctica	*Cardiospermum grandiflorum*
Cissus hypoglauca	*Cassia spp.*
Clematis anistata	*Cayratia clematidea*
Commelina cyanea	*Centella asiatica*
Corybas acontiniflorus	*Chrysanthemoides monilifera*
Corybas pruinosus	*Cissus antarctica*
Cryptocaria spp.	*Commelina cyanea*
Cyperaceae	*Conyza spp.*
Desmodium varians	*Crassocephalum crepidioides*
Dianella spp.	*Cryptocaria spp.*
Dichondra repens	*Cupaniopsis anacardioides*
Dioscorea transversa	*Cuscuta spp.*
Doodia aspera	*Cyperaceae*
Endiandra sieberi	*Dactyloctenium australe*
Eustrephus latifolius	*Dianella spp.*
Fabaceae	*Dichondra repens*
Ficus coronata	*Dioscorea transversa*
Geitonoplesium cymosum	*Ehrharta erecta*
Geranium homeanum	*Entolasia marginata*
Ghania aspera	*Erigeron bonariensis*
Ghania melanocarpa	*Euphorbia spp.*

Glycine microphylla
Goodenia ovata
Guioa semiglauca
Gymnostachys anceps
Hibbertia dentata
Hibbertia scandens
Homalanthus populifolius
Hydrocotyle tripartita
Hypolepis muelleri
Imperata cylindrica
Jacksonia scoparia
Lantana camara
Legnephora moorei
Lepidosperma laterale
Leucopogon ericoides
Leucopogon lanceolatus
Liliaceae
Livistona australis
Lomandra longifolia
Marsdenia spp.
Morinda jasminoides
Myrsine howittiana
Myrsine variabilis
Notelea spp.
Oplismenus aemulus
Oplismenus hirtellus
Oxalis perennans
Pandorea pandorana
Parsonsia straminea
Passiflora subpeltata
Pellaea paradoxa
Pittosporum multiflorum
Pittosporum revoltum
Plectranthus parvifolius
Podolobium ilicifolium
Polystichum australiense
Pratia purpurascens
Pseuderanthemum variabile
Psychotria loniceroides
Pteridium esculentum
Pterostylis spp.
Rhodamnia rubescens
Ripogonum album
Ripogonum spp.
Eustrephus latifolius
Fabaceae
Ficus coronata
Geitonoplesium cymosum
Ghania melanocarpa
Glochidion ferdinandi
Glycine_microphylla
Guioa semiglauca
Homalanthus populifolius
Hydrocotyle tripartita
Imperata cylindrica
Ipomoea cairica
Juncus spp.
Lantana camara
Livistona australis
Lomandra longifolia
Microlaena stipoides
Murraya paniculata
Nephrolepis cordifolia
Notelea spp.
Ochna serrulata
Oplismenus aemulus
Oplismenus hirtellus
Oxalis spp.
Pandanus spp.
Pandorea pandorana
Plectranthus parvifolius
Pteridium esculentum
Rhodomyrtus psidioides
Ripogonum spp.
Rubus moluccanus
Sarcopetalum harveyanum
Senna spp.
Smilax glyciphylla
Solanum nigrum
Stenotaphrum secundatum
Stephania japonica
Synoum glandulosum
Tradescantia
Viola hederacea

Rubus parvifolius
Sarcopetalum harveyanum
Senna spp.
Smilax glyciphylla
Stellaria media
Stephania japonica
Synoum glandulosum
Tetrastigma nitens
Trochocarpa laurina
Veronica plebeia
Viola hederacea
Wahlenbergia spp.

Fig A12 *Rhodamnia rubescens* **a)** dead adults at Royal National Park; **b)** fungicide spray equipment; **c)** plot showing treatment with plastics to avoid fungicide from dripping on the understory; **d)** infected leaf showing yellow pustules; **e)** infected flower buds; **f)** non-infected flowers; **g)** non-infected fruits; and *Rhodomyrtus psidioides* **h)** infected seedling.